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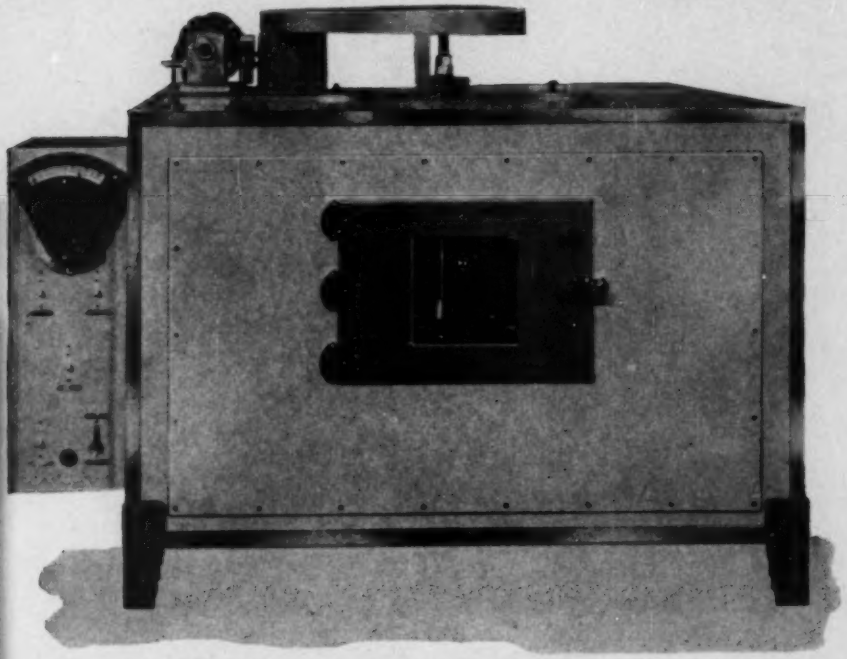

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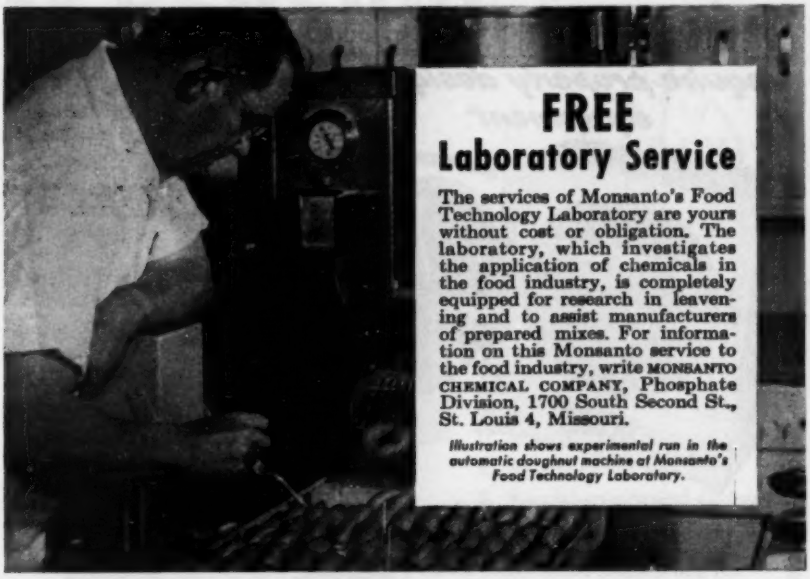

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CEREAL CHEMISTRY

VOL. XXVIII MARCH, 1951 NO. 2

AN INSTRUMENT FOR ESTIMATING THE MOISTURE CONTENT OF GRAIN AND OTHER MATERIALS BY THE MEASUREMENT OF ELECTRICAL CONDUCTANCE[1]

C. F. BROCKELSBY[2]

ABSTRACT

A fully portable instrument for laboratory and field use is described; it measures conductance in a circuit developed from the Wheatstone bridge. The grain is ground before testing. In grinding wet grain by the recommended method, the loss of moisture is small and independent of the atmospheric conditions for water vapour pressure differences, between grain and atmosphere, up to at least 15 mm. Hg; the usual empirical calibration procedure, therefore, automatically compensates for this loss. The meal is compressed in the cell; the exact quantity is unimportant and the sample is tested at substantially ambient temperature. The instrument has been calibrated by the makers and the National Institute of Agricultural Engineering on 204 samples of English wheat from the 1946 to 1949 harvests. The standard error over the moisture range 12 to 22% is 0.34%, falling to 0.29% on eliminating the variance between localities. Inequalities of moisture distribution such as those produced by a British farm drier have a negligible effect. Calibrations for conditioned and imported wheats, flour and other mill stocks have been made by the makers and Henry Simon Ltd; the instrument has also been tested by the Research Association of British Flour Millers. There are systematic differences up to 0.3% moisture between wheat types. Conditioning produces a bias of about 0.3% moisture content in the direction of the bran moisture. The effect of bushel weight is negligible. Wheat tested immediately after wetting with cold water gives high readings; for soft wheat, the error is less than 1% and vanishes in two hours; for hard wheat, the error is 1% after four hours and vanishes in 24 hours. The experimentally determined temperature correction is 0.104 ± 0.002% moisture content/°C. The performance on other cereal grains resembles that on wheat; calibrations, usually with a standard error of about 0.2%, have also been made for other hygroscopic materials.

Instruments for estimating moisture content by means of electrical measurements have long been available, but none is entirely satisfactory for field use, especially in a maritime climate. In those operating by the measurement of capacitance, it is necessary to weigh

[1] Manuscript received November 15, 1949.

[2] Physicist, Marconi Instruments Ltd., St. Albans, England.

out the sample if sufficient accuracy is to be attained in spite of variation in bushel weight. Previous instruments using the measurement of conductivity and giving sufficient accuracy without a weighing are not portable enough for convenient use in the field. A more important defect of previous instruments is the large error caused by any departure from uniform moisture distribution in the kernels. Any electrical test on whole grain is strongly biased by the state of the superficial layers. In a maritime climate, such as prevails in Great Britain and neighbouring countries, the moisture in the harvested grain is usually unevenly distributed. Much of the grain is too damp to store and must be artificially dried, which produces steep moisture gradients within the kernels. An instrument for sorting damp grain in the field and for dryer control must give sufficiently accurate readings on such grain.

The new meter[3] was, therefore, designed to be completely portable, and of adequate accuracy even on freshly dried samples and those newly harvested in unfavourable weather. The applications in flour milling and to granular, powdery and fibrous materials in general were also given due weight.

Principles of operation

The electrical conductance and capacitance are both roughly proportional to the density of the material in the cell, but the rate of change of conductance with moisture content is two orders of magnitude greater than that of capacitance, so that the density is relatively unimportant. Both electrical methods are capable of an adequate accuracy, but the apparatus for conductance measurement is simpler in principle. A measurement of conductance was therefore adopted.

Because the electrical properties of whole grain are preferentially affected by the moisture content of the surface layers, the kernel structure is destroyed by coarsely grinding before the test. In the cell the meal is compressed against two coplanar electrodes; the electric test current penetrates the samples only to a depth of the order of the electrode separation, so that the quantity of meal in the cell is unimportant above a certain minimum. Large differences between the grain and the ambient temperatures are much reduced by grinding and the meal rapidly assumes the cell temperature, which is usually 1–2°C. above ambient; temperature corrections are therefore based upon ambient temperature.

Electrical and Mechanical Details

The electrical measuring circuit (Fig. 2) is a development of the Wheatstone bridge. One arm includes a thermionic tube and this arm

[3] Marconi Instruments Ltd. Type no. TF 933.

is itself in the form of a subsidiary Wheatstone bridge. The initial adjustment consists in balancing the subsidiary bridge, thus bringing the tube into a standard state irrespective of its precise characteristics and of the supply voltages. An international octal-based tube is used and standard British and U.S. makes are interchangeable. Power supply is from internal dry batteries; types which are standard throughout the world are employed. The life of the A cell exceeds 150 working hours; that of the B battery is several times longer. The battery space may, alternatively, be filled by a supply unit operating from 100 to

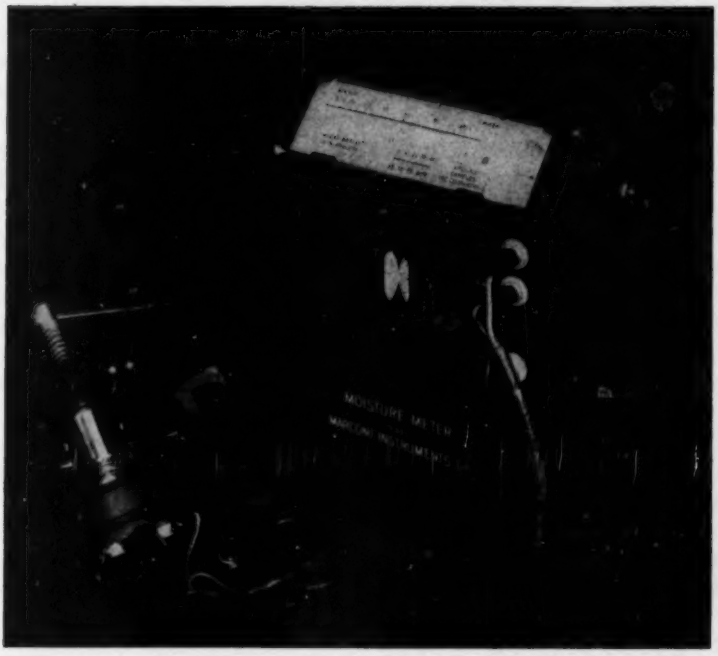

FIG. 1. General view of the instrument. The C-clamp applies a standardized pressure to the sample through a calibrated spring housed in the cylindrical part.

125 and 200 to 250 v. alternating current at 40 to 100 cycles per second.

The appearance of the instrument is shown in Fig. 1. The test cell, on the left, is based on a C clamp. The sample is tested under a standardized pressure of 1,000 lb./sq. in., applied by means of the tommy bar, which can be turned with one finger. The calibrated spring is pre-stressed so that the thrust is applied by only a half turn of the screw. The cell and clamp stow in a compartment at the back of the instrument case, where there is also space to carry at least one

suitable type of hand grinder. The scale, visible at the top of the photograph, is a slide rule which converts the instrument reading (taken through the small window in the front) into moisture content and simultaneously corrects for temperature. The sliding scale clips on, so that calibrations for various substances can be fitted. Each scale carries two calibrations and those not in use can be stored in the back compartment.

The over-all dimensions are 12 × 10 × 7½ in. and the weight, including the cell, clamp and batteries, is 14 lb.

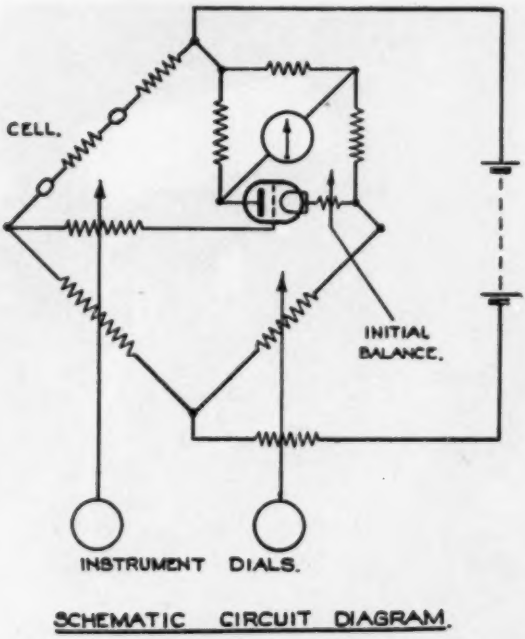

FIG. 2. Electrical circuit. One arm of the Wheatstone bridge, containing the tube, is a subsidiary Wheatstone bridge which is balanced in the preliminary adjustment of the apparatus.

Grinding. There is an *a priori* probability that the moisture content will change during grinding in an atmosphere with which the wheat is not in hygroscopic equilibrium. A change which is independent of, or varies systematically with, the moisture content does not matter; it will be taken up in the instrument calibration. It is, however, clearly desirable to minimize the change. Experiments made in search of a satisfactory method showed that a loss of 1% in moisture can occur in wheat with an 18% moisture content when ground very finely or in a mill which causes considerable heating, but the loss is

small if the mill runs cool and produces only a moderately fine meal. A small coffee mill with two fluted cones is suitable when set so that less than 60% of the product passes 30 mesh. Excessively coarse grinding defeats the object of the process; 35% should pass 30 mesh.

The following investigation of the effect of grinding is extracted from the results of a wider comparison between methods of moisture determination in different laboratories. Five of these used mills like the above, all of different sizes and of four different makes. One laboratory used a similar mill set very fine, and one a powerful multi-crown laboratory mill. Moisture determinations were made, with direct grinding and with preliminary drying, on four wheat samples, Manitoba and English, each having a moisture content of approximately 12 and 19%.

In the five laboratories, a total of nine such pairs of determinations were made on each sample, using three oven methods: four hours at 120°C., 16 hours at 100°C. and the Carter-Simon.[4] The mean increases in apparent moisture produced by predrying were: Manitoba: dry, −0.03%; damp, 0.01%; English: dry, 0.02%, damp 0.13%. Only the last is significant; the standard deviation of the mean of nine "increases," estimated from the error variance, was 0.038%. The relative humidity during grinding was about 50%. In the other laboratories, fine grinding in a coffee mill caused a loss in moisture of about 0.6% on the damp samples; the multi-crown mill caused a loss in moisture of about 1.5% on all four samples.

If the loss in grinding is proportional to the difference between the water vapor pressure in equilibrium with the wheat and that existing in the atmosphere during grinding, the above result suggests that serious errors could result from grinding in hot, dry atmospheres. A small supplementary experiment was therefore made. English wheat samples containing about 16 and 20% moisture were tested in the four hour oven with preliminary drying and with direct grinding in atmospheres of approximately (i) 20°C., 80% r.h., (ii) 20°C., 50% r.h., (iii) 29°C., 40% r.h. For the drier sample, none of the results differed significantly from the over-all mean value of 15.7% moisture content. The vapor pressure differences between the sample and atmosphere were about 1, 4 and 11 mm. Hg. The wet sample gave 19.7% with preliminary drying and the apparent grinding moisture losses in the three atmospheres were 0.32, 0.24 and 0.28%, with vapor pressure differences of about 2, 7, and 15 mm. Hg. The loss appears independent of the vapor pressure difference; it will, therefore, be taken up in the calibration and will not produce an error when the ambient conditions during

[4] The Carter-Simon oven is an electrically heated thermostatic instrument having a small tunnel through which standard-sized (2½ in.) aluminum pans are pushed at five minute intervals, giving a total drying time of fifteen minutes. Preheated air at 155°C. is drawn through the tunnel by a chimney.

use differ from those during calibration. For hard wheats, the loss in grinding is certainly smaller than for soft, probably because much less work is done on the grain during grinding. If it were to become necessary to make measurements of high accuracy on soft wheat at more than, say, 17% moisture content, in atmospheres of very widely varying temperature and humidity, it would be advisable to investigate the loss in grinding more fully.

The required grinding takes about the same time (30 seconds) as weighing out a sample on a robust, direct-reading, aperiodic balance.

Calibration for English Wheat

The instrument has been calibrated by tests, described below, on wheat from the harvests of 1946–1949 inclusive, against two oven methods, using three instruments, in the field, laboratory, and flour mill. The combined results of all the tests which fell in the range 12 to 22% moisture content (204 points) are shown in the scatter diagram, Fig. 3, where the instrument reading is plotted on a scale of "approximate moisture content." The regression equation is:

$$\text{Instrument moisture content} = 1.078\ (\text{oven moisture content}) - 1.20.$$

The standard deviation about the regression is 0.34%. The differences between seasons, varieties and treatments and between field and laboratory tests are not significant; the variance "between localities" is significant; eliminating it reduces the standard deviation about the regression from 0.34 to 0.29%.

Preliminary tests were made in the company's laboratory early in 1948, using an experimental instrument and the 4 hour 120°C. air oven on 37 samples from the 1946 and 1947 harvests.

A pre-production prototype instrument was tested in the field by the National Institute of Agricultural Engineering (3) through the harvest of 1948. The results contribute 38 points to Fig. 3. Samples were taken directly from the combine harvester, from storage bins and sacks, and from the farm drier input and output. The instrument test was usually made on the spot, within a few minutes of drawing the sample, but some were made a few hours later or on the next day. The samples were kept in sealed jars for subsequent test in the Carter-Simon oven. A calibration based on these tests was fitted to the instruments supplied to farmers in 1949.

Tests against the 4 hour 120° air oven were made, at periods of a few hours to a few months after harvesting, on 118 samples (supplied by Mr. Gardner, of the Hertfordshire Institute of Agriculture, and Mr. Greer of the Research Association of British Flour Millers) from a 1949 trial in which nine varieties of wheat were grown in four localities with three manurial treatments.

Wheat samples from a flour mill were tested in 1949; the results on eleven "English intakes" are included in Fig. 3.

Wet Wheat. The National Institute of Agricultural Engineering tests included some samples at moisture contents over 22%, at which English wheat cannot be ground; it is too soft and sticky. Instrument readings were taken on whole grain. The instrument can be used in this way for rough tests up to moisture contents of 25 or 26%.

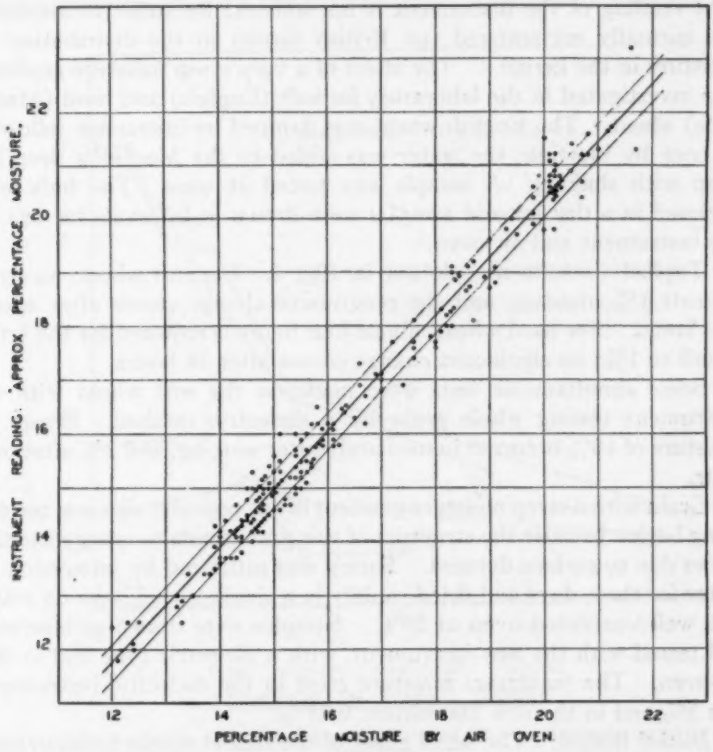

FIG. 3. Scatter diagram showing the results of 204 tests on English wheat from the harvests of 1946–1949.

Spoiled Wheat. The N.I.A.E. tests included nine "very green and dirty" samples from a crop which had been laid by storm and left lying until the end of the harvest. The oven moistures, before and after drying, covered 16 to 22% moisture; the instrument readings (not plotted in Fig. 3) were low by 0.7 to 1.4%, the average being 1%. Samples of sound grain from the same farm gave an average error of −0.3%. The systematic error attributable to the spoiling of the grain

is thus about −0.7%. In their report (3) the N.I.A.E. recommended a slightly modified calibration which brought all the readings at moisture contents up to 20%, including those on the spoiled wheat, within ±1%; it was considered possible that the deviations were due not to spoilage but to some other cause. The results of the later tests convince the writer that this possibility can be ruled out.

Moisture Gradients. One object of the N.I.A.E. field tests was to find the effect of moisture gradients; their report concludes, *inter alia*, "the reading of the instrument is not affected by such variations as are normally encountered (on British farms) in the distribution of moisture in the kernel." The effect of a very steep moisture gradient was investigated in the laboratory for soft (English) and hard (Manitoba) wheat. The English wheat was damped by immersion followed at once by blotting; the water was added to the Manitoba drop by drop with shaking. A sample was tested at once. The bulk was enclosed in a dry jar and samples were drawn at intervals for test in the instrument and air-oven.

Typical results are plotted in Fig. 4. For soft wheat, no error exceeds 1% moisture and the progressive change ceases after about two hours. For hard wheat, about four hours is required for the error to fall to 1%; no significant change occurs after 24 hours.

Some simultaneous tests were made on the soft wheat with an instrument testing whole grain by a dielectric method. Errors in moisture of 10% occurred immediately after wetting, and 2% after one hour.

Grain with a steep moisture gradient of the opposite sign was tested, using barley because the structure of this grain tends to exaggerate the errors due to surface dryness. Barley was saturated by immersion in water for three days and dried rapidly in a single-kernel layer on trays in a well ventilated oven at 50°C. Samples were drawn at intervals and tested with the new instrument, with a dielectric type and in the air-oven. The maximum moisture error in the dielectric instrument was 5% and in the new instrument 0.35%.

Bushel Weight. The small effect of the bushel weight is illustrated by the following tests. A sample in which one quarter of the kernels had been attacked by weevils so as to reduce the average weight to one half of that of a sound kernel was tested in the oven, by a dielectric meter and by the new instrument. The dielectric instrument read 2.3% low with no density correction, but only 0.4% low with density correction. This tends to confirm the opinion, formed on general grounds, that reduced density due to weevil attack produces much the same effect on an electrical instrument as a naturally low density in the grain. The new meter gave an error in moisture of −0.2%.

The addition of 5% by weight of cocksfoot seed to a good, clean sample of wheat at a 14.3% moisture content reduced the bushel weight by nearly 10% and produced an error of 2% in the dielectric meter, but of only 0.2% with the new instrument.

Temperature. The effect of temperature was determined by direct experiment; the resulting coefficient proved satisfactory when used to correct the readings taken through the calibration work.

Eighteen samples of clean English wheat were chosen with moisture contents spread over the range 10 to 20%. Instrument readings at 5

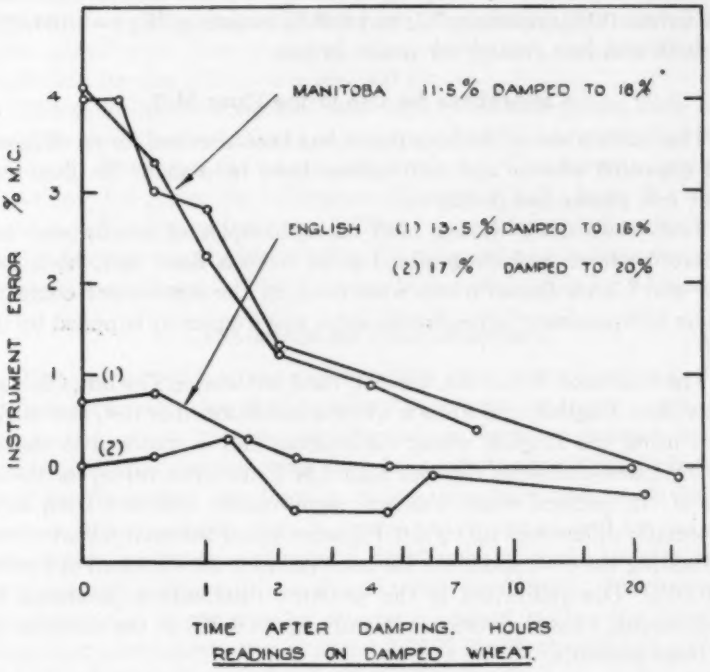

FIG. 4. Tests on recently wetted wheat, showing the marked difference in behavior between soft and hard types.

to 30° C. were obtained by three methods. (i) Readings were first taken at normal laboratory temperature. The instrument, grinder and samples were then cooled to 5°C. in a cold room and readings were taken. Repeat tests were made at normal temperature. (ii) A cell was modified to permit the insertion of a thermometer bulb into the metal pressure plunger, and was lagged. This cell was heated to about 35°C., loaded with meal and quickly closed. Instrument reading and temperature were recorded frequently for the next 30–40

minutes while the cell cooled, the electric test potential being applied for the shortest time needed to take each reading. An excellent straight-line relation between instrument reading and temperature was found for readings taken after the first few minutes. A few tests were rejected because extrapolating this line to room temperature revealed a change of moisture content. (iii) Method (ii), but with the cell initially cooled to 0°C.

The results were expressed as rate of change of apparent moisture content with temperature. The mean coefficient was 0.104 ± 0.002% moisture/°C.; it did not vary significantly with the moisture content. The values 0.1% moisture/°C. and 0.06% moisture/°F. (=0.108/°C.) are both accurate enough for practical use.

Calibrations for Use in the Flour Mill

The calibration of the instrument has been checked for conditioned and imported wheats and calibrations have been made for flour and other mill stocks and products.

Tests were made during 1949 on 68 samples of conditioned and imported wheat, including mixed grist, from a flour mill; both four hour and Carter-Simon ovens were used by the instrument company and an independent Carter-Simon value was frequently supplied by the mill.

The results on Manitoba, Garnet, hard red winter, Durum, Chilean, Australian, English, and French wheats, conditioned or not, and mixed grist, using the English wheat calibration, had a standard deviation of 0.31% moisture from the four hour 120°C. air oven value; the deviation of the general mean was not significantly different from zero. Systematic differences up to ±0.3% were found between wheat types; eliminating the corresponding variance reduced the standard deviation to 0.26%. Disequilibrium in the moisture distribution, produced by conditioning, caused errors in moisture up to 0.3% in the direction of the bran moisture.

The instrument company's results by the Carter-Simon method showed random differences from the four-hour result comparable with those of the moisture meter, with standard deviation of 0.29%. Significant differences were found, with standard deviation 0.17%, between wheat types; the net standard deviation within wheat types was 0.26%. The mill Carter-Simon results showed no systematic difference from the company's results by the same method; the standard deviation of one oven test, assuming the errors to be due equally to both, was 0.25%.

The instrument was briefly tested by Greer (2), of the Research

Association of British Flour Millers, in 1949, through a fortnight of general laboratory work; 50 samples of wheat, mainly English but some Manitoba, were tested, including some which had been conditioned with water, or in steam at 130°C., for laboratory milling tests; white flour (65% extraction) and bran from these tests were also used. Greer concluded that the difference between the instrument and oven estimation is unlikely to exceed a moisture content of 0.5%.

Tests (1) were also made in 1949 by Messrs. Henry Simon Ltd. (the sole agents for the instrument in the cereal milling field) on English and imported wheat, National flour (85% extraction), mill flours of high and low grade, imported flour, semolina, bran and germ. These tests agreed with those reported above for wheat and provided the main data for the calibrations for mill stocks.

The calibration for flour of very high extraction differs little from that for wheat. The calibrations for bran and for low extraction flour (as C roll) differ from the wheat scale by about 1% moisture in opposite directions; for germ, the calibration diverges rapidly from the wheat scale as the moisture increases. Other materials, including National (85%) flour, give calibrations related in a logical way to those for bran, endosperm and germ. The accuracy is similar to that for wheat.

Calibrations for Other Materials

The instrument has been calibrated for many other materials. The performance resembles that on wheat when testing barley, oats, rye, and maize; a few tests on cereal products such as pinhead barley, groats, and rolled oats suggest that their calibrations differ little from those for the grain. Preliminary calibrations have been made for the seeds of several grasses, trefoils, beets, and brassicas, and for pea and bean flours, including soya, copra, dried seaweed and alginates, gelatine, glue and isinglass, fish meal, and whale meat meal. Mixtures like provender and gravy powder require a special calibration for each blend, but a standard error of about 0.25% is still obtained in spite of the oil and mineral content.

Calibrations have been made for many fibrous materials including cotton, jute, wool and rayons, chemical wood pulp, paper and cardboard. Some of these can be tested in the bale by using "sword" electrodes. The instrument has also been calibrated for cigarette tobacco.

Acknowledgments

Acknowledgment is due to Marconi Instruments Ltd. for permission to publish this paper. The author would also like to thank Mr. Hamblin of the National Institute of Agricultural Engineering, Mr. Greer of the Research Association of British Flour Millers, and Mr. Dinsdale of Henry Simon Ltd. for their continued

interest and co-operation. Most of the instrument readings and oven moisture determinations made in the company's laboratories were carried out with unfailing accuracy by Mr. P. J. Crumpler.

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SOME EFFECTS OF SOLUBLE FLOUR COMPONENTS ON BAKING BEHAVIOR[1,2]

JAMES W. PENCE, ANGELINE H. ELDER, AND DALE K. MECHAM

ABSTRACT

Glutens and components soluble in 0.1%, pH 6.8 phosphate buffer were separated from five flours of widely differing baking characteristics, and baking results were obtained with micro-doughs reconstituted with various combinations of these preparations and a single lot of starch.

The soluble components of the flours were found to be required for maximum performance of all the glutens except that from a durum wheat. Less variation was found among unsupplemented glutens than among glutens supplemented with solubles from a single flour or among solubles used with a single gluten. The soluble components thus appear capable of affecting flour behavior to such an extent that differences or similarities in gluten characteristics may be obscured.

The soluble fractions were further separated into a dialyzable and a non-dialyzable fraction. The soluble part of the latter, designated crude albumin, consistently produced a positive volume response and shortened mixing time when added to gluten-starch doughs. The dialyzable fraction produced a smaller volume response, in general, and tended to increase mixing time. The substantial pentosans content of the crude albumin fraction was found to be responsible for the shortened mixing time of doughs containing this fraction. The high sugar and salts content of the dialyzable fraction appeared to be responsible, at least partly, for the effects observed with this fraction because similar volume and mixing effects were obtained by adding extra sucrose and salt to gluten-starch doughs.

Proteins from other sources, as well as other wheat proteins, were not as effective as the flour albumin for supplementing gluten.

The observations of several investigators suggest that the contribution of soluble flour constituents to baking characteristics may differ rather widely among flours. For example, Sandstedt, Jolitz, and Blish (13) reported similarity between loaves reconstituted with gluten and

[1] Manuscript received July 10, 1950. Presented at Annual Meeting, May 1950.
[2] Contribution from Western Regional Research Laboratory, Albany, Calif.
Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

starch, omitting the solubles, and loaves baked from the original flour. In contrast, Finney (6) found that omission of solubles from reconstituted doughs resulted in a definite decrease in loaf volume with two flours but had no effect with a third. Baker, Mize, and Parker (2) found that doughs containing more than the normal level of solubles gave loaves of increased volume. Ofelt (10) obtained a slightly lowered loaf volume with certain baking procedures when solubles were added to reconstituted doughs. Elder, Pence, and Mecham (5) reported that addition of solubles to starch-gluten doughs from several flours gave a varied but generally large positive volume response.

These variations in effects of soluble constituents appear sufficiently large to mask differences or similarities in the baking potentialities of glutens when comparisons are attempted by baking with whole flours, and suggest a partial explanation for the failure of earlier efforts to show a satisfactory correlation between baking characteristics and amount or chemical composition of the gluten proteins of flours.

Much of the variation in results among the papers ctied might be attributed to differences in the fractionating and baking procedures used rather than to differences in the soluble constituents of the various flours. The work reported in the present paper was undertaken to obtain additional information regarding the effects on reconstituted doughs of solubles, and solubles fractions, prepared under standardized conditions from flours of widely differing characteristics. It was found that, with the procedures used in this work, the addition of flour solubles to gluten-starch doughs consistently gave a marked positive volume response. Much of this effect was found to be due to a crude albumin fraction of the solubles.

Materials and Methods

The unbleached, straight-grade flours used in this work were milled from lots of pure varieties of wheat selected to represent widely differing types as shown in Table I.

TABLE I

DESCRIPTION OF THE FLOUR SAMPLES[1]

Variety	Where Grown	Type	% Protein[2]
Baart	Washington	White Spring	9.5
Turkey	Kansas	Hard Red Winter	10.2
Red Chief	Kansas	Hard Red Winter	10.3
Pentad	North Dakota	Durum	11.6
Thatcher	Montana	Hard Red Spring	14.2

[1] Additional information on the preparation, composition, and baking properties of these flours is given by R. K. Durham, *Transactions*, American Association of Cereal Chemists, Jan. 1951, under the title "Properties of Flours Milled from Selected Wheat Varieties."
[2] 14% moisture.

The flours were separated into glutens, total solubles, and starches by washing doughs in the 0.1%, pH 6.8 phosphate buffer of Dill and Alsberg (3). The wash solutions were kept ice-cold during washing, and a fixed ratio of buffer to flour was used. Glutens were frozen in thin sheets on dry ice and stored in sealed containers at −30°F. after being broken into small pieces. Starch was separated from the wash solution by means of a refrigerated centrifuge, and the supernatant liquid was vacuum-concentrated below 40°C. to a thick syrup which was dried by lyophilization. Standard starch was substituted for the flour starches in reconstituted doughs to eliminate variability from this source. The large lot of standard starch was prepared from flour in a pilot-plant operation using the batter process (4).

The formula and bread-baking procedures recommended by Finney and Barmore (7) were used with only minor modifications. The shortening and non-fat milk solids were used as recommended, but 0.25% malted wheat flour was substituted for the malt syrup. Optimum levels of bromate were used for each of the original flours, and the same amounts were added to the corresponding reconstituted doughs. Ammonium chloride was added to the formula to minimize the effects of variations in gas production, especially during the critical pan-proof period, as recommended by Ofelt and Sandstedt (11) and by Walden and Larmour (14). Satisfactorily uniform gassing rates were obtained with additions to the formula of 0.05 to 0.25% of ammonium chloride, depending upon the type of reconstituted dough.

Micro-doughs from 30 gm. of flour, or the equivalent of flour fractions, were mixed to optimal dough development with a National Recording Mixograph.[3] The doughs were punched with sheeting rolls adapted to small doughs and were molded by hand. Micro-pans were constructed according to scaled-down dimensions of the standard A.A.C.C. low-form, pup-loaf pan. Loaves were baked 20 minutes at 430°F.

Doughs were reconstituted to correspond to the use of flour containing 12% protein at 14% moisture. The ratio of soluble to gluten protein to be used was determined from the amount of total flour nitrogen removed in washing out the gluten. The same quantities of gluten and standard starch were then used for all doughs reconstituted from each fractionation of a flour, and the soluble fractions were added or omitted according to the desired experiment.

Effects of Total Buffer Solubles on Baking Behavior. Previous papers from this laboratory (5, 12) have reported a consistent positive volume response of gluten-starch doughs to the inclusion of total solu-

[3] Mention of trade names of materials and equipment does not constitute endorsement by the Department of Agriculture.

bles. Results confirming and extending these findings are shown in Table II for the five flours used in this study.[4] The responses shown in the last column were all positive, with the exception of the durum flour, and ranged from 0 to 25% of the volumes of the loaves containing the total solubles, the lower responses being obtained with the flours unsuitable for bread production. From a total of 11 widely varying flours thus far tested in this manner, only two zero responses (from a Red Chief and the durum wheat) and no negative responses have been observed.

Results in the first two columns afford an approximate comparison of glutens from various flours at nearly equal protein contents. Sur-

TABLE II

EFFECT OF SOLUBLES ON LOAF VOLUMES OF GLUTEN-STARCH DOUGHS FROM DIFFERENT FLOURS

Variety	Amount of Gluten Used[1]	Volume of Gluten-Starch Loaf	Volume of Gluten-Starch-Solubles Loaf
	g.	*cc.*	*cc.*
Baart	3.18	151	201
Turkey	3.18	157	209
Red Chief	3.21	151	177
Pentad	3.09	139	141
Thatcher	3.21	155	198

[1] Doughs contained 3.60 gm. total protein when fully reconstituted. Ratio of gluten to soluble protein used determined by amount of soluble protein recovered when washing out glutens.

prisingly little variation is shown except for the gluten from the durum wheat flour. A strict comparison is not possible because the different flours contain different ratios of soluble to gluten protein so that different amounts of gluten were required for a 12% protein dough; in addition, completely uniform separation of the non-gluten proteins was not always obtained with the procedures used. Soluble protein recoveries from the various flours ranged from 10.6 to 13.8% of the total flour nitrogen, with most of this variation reflecting actual differences in soluble protein content. The results in column 3 rank the flours more nearly in agreement with expected performance and show that the baking quality of the Baart proteins is good when the protein level is high enough.

Variations Among Total Solubles. Experiments described in the preceding section showed that different gluten-starch doughs varied in their response to addition of their corresponding solubles. Such a result could be attributed to variations among glutens in their ability to respond to solubles, rather than to variations among the solubles.

[4] Whenever possible doughs were baked in triplicate. Statistical treatment of results from this study indicated that a difference of 8 cc. between mean loaf volumes is required for significance at the 5% point.

Experimental evidence of variations among total solubles themselves was obtained by baking the series of five solubles preparations with two different glutens.

These results (Table III) appear to be interpretable only as showing that both glutens and solubles vary among flours. The Thatcher gluten was able to give a satisfactory loaf volume if supplemented by any of the five solubles preparations, with little spread in final volume. The Red Chief gluten, on the other hand, was capable of giving a reasonably large loaf volume only if supplemented by suitable solubles.[5]

TABLE III

LOAF VOLUMES OF VARIOUS GLUTEN-STARCH DOUGHS RECONSTITUTED WITH SOLUBLES FROM THE VARIOUS FLOURS

Variety	Loaf Volume		
	Various Glutens with Thatcher Solubles	Thatcher Gluten with Various Solubles	Red Chief Gluten with Various Solubles
	cc.	cc.	cc.
Baart	170	199	194
Turkey	192	205	193
Red Chief	179	196	187
Pentad	159	194	167
Thatcher	196	198	185

Significant, though small, variations among the solubles in this regard are thus shown. It is quite possible that each gluten requires a minimum amount of certain solubles components for maximum performance, and since the individual components are not likely to occur in equal amounts in different flours, it is reasonable to expect variations among solubles preparations on quantitative grounds without consideration of qualitative differences.

Effects of Solubles Fractions on Baking Behavior. Since it was shown in a previous paper (12) that the volume response to added solubles can be attributed to their nitrogenous components, it was decided to determine whether fractions of these components could be responsible for some of the effects shown by the total mixture. About one-fourth of the nitrogen in the total solubles will diffuse through a cellophane membrane. Accordingly, dialysis was used to separate low molecular weight materials from protein components of the solubles.

Three fractions were obtained by dialysis of the buffer solubles—(a) the dialyzables, containing sugars, low molecular weight nitrogenous compounds, and buffer and flour-ash salts; (b) a crude albumin

[5] Results reported previously (5) include the pup-loaf volumes of 840, 800, 775, and 775 cc. for a Turkey gluten baked with Turkey, Baart, Red Chief, and Pentad solubles, respectively.

fraction, containing water soluble proteins and a considerable amount of pentosans (about 30–40% of the dry weight); and (c) a relatively small "globulin" fraction consisting of protein material insoluble in water. Although referred to as a "globulin" fraction, analytical and solubility characteristics of the latter fraction make it more likely to be a mixture of denatured albumin and gliadin, with little true globulin present.

The results of baking tests showing the responses of the different glutens to such fractions are presented in Table IV. The effect of the

TABLE IV

LOAF-VOLUME RESPONSES OF DIFFERENT GLUTEN-STARCH DOUGHS TO THE ADDITION OF CORRESPONDING SOLUBLES FRACTIONS

Variety	Loaf Volume Response After Additions to Gluten-Starch Doughs			
	Total Solubles	Crude Albumin	Dialyzables	Dialyzables Plus Albumin
	cc.	cc.	cc.	cc.
Baart	50	11	18	35
Turkey	52	39	9	28
Red Chief	26	20	9	28
Pentad	2	7	0	−4
Thatcher	43	20	19	48

total solubles was recovered nearly completely in the sum of albumin and dialyzables responses (column 3 plus column 4) except with the Baart flour. Nothing more than an additive effect was found when albumin and dialyzables were added to the same dough; in fact with two of the flours (Turkey, Pentad) less than an additive effect was obtained. No explanation of these small responses and the low response of Baart gluten to albumin can as yet be offered except that the baking test conditions may have been inadequate for these particular doughs. The "globulin" fraction referred to above was without effect.

Since some proteolytic or oxidative changes could easily have occurred in either or both the albumin and dialyzables during dialysis and concentration, the approximate recovery of the total solubles effect appears to be all that could reasonably be expected. The albumin fraction, for instance, was found to be quite easily denatured; insoluble material would separate from solution on standing, and lyophilized preparations were not always completely redissolvable in water. Presumably, the insoluble material is less effective for supplementing gluten because heat-denatured albumin was found to have lost completely such ability. Baking results demonstrating this are shown in Table V. The response to the heated total solubles was reduced to

that imparted by the dialyzable portion of the fraction, and heated albumin was completely without effect.

Although the above-described fractionation did not give completely satisfactory results with all the flours (Table IV), the baking values do indicate that the crude albumin fraction was generally responsible for most of the positive volume response given by total solubles. The volume responses to the dialyzable fractions were generally smaller than those to the albumin fraction, but with two of the glutens (Baart, Thatcher) they appear large enough to be of significance in the comparison of flours.

In addition to the loaf volume responses of the albumin and dialyzables fractions, a distinct effect of these fractions on mixing behavior of reconstituted doughs was observed. Crude albumin decreased the mixing time of gluten-starch doughs and restored normal handling characteristics to the doughs. Dialyzables, on the other

TABLE V

EFFECT OF HEAT DENATURATION[1] OF ALBUMIN AND TOTAL SOLUBLES ON LOAF VOLUME RESPONSES OF GLUTEN-STARCH DOUGHS (THATCHER GLUTEN)

Additions to Gluten-Starch Doughs	*Loaf Volume*
	cc.
None	157
Total solubles	198
Heated total solubles	171
Dialyzables	174
Albumin	186
Heated Albumin	154

[1] Heated 30 minutes on steam bath in aqueous solution.

hand, appeared to soften the surface of the wet gluten particles, delaying dough development by interfering with their coherence unless the absorption of the doughs was decreased by about 4%.

Previous observations (12) have shown that the pentosans of wheat flour restore normal handling characteristics to gluten-starch doughs; it therefore seemed probable that the high pentosans content (30–40%) of the crude albumin fraction was responsible for the mixing effects observed with this fraction. Likewise, it seemed that the high sugar and salts content of the dialyzable material might be associated with the mixing and baking effects observed with this fraction. In Table VI are included typical mixing and baking results obtained with gluten-starch doughs to which were added either purified albumin[6] containing only small amounts of pentosans, an amount of sucrose equal to the total quantity of dialyzables normally used, or an amount of salt equal

[6] Fractional precipitation with ammonium sulfate was used to reduce the pentosans content of albumin preparations to less than 5%.

to that estimated to be contained in the quantity of dialyzables normally used.

These results show the marked effect of pentosans or pentosans-containing fractions on the mixing behavior of gluten-starch doughs and the similarity of the effects of sucrose and salt to that of the dialyzables on the mixing behavior of such doughs. With regard to the large loaf volumes obtained with the sugar-salt doughs, it should be pointed out that the quantities used exceed the amounts of sucrose actually contained in the dialyzables fraction and may have exaggerated the baking effects. The extra sucrose added to these doughs raised the sugar content of the doughs to about 10% of the weight of flour used;

TABLE VI

EFFECTS OF ADDED CRUDE OR PURIFIED SOLUBLES FRACTIONS, SUCROSE, OR SUCROSE AND SALT ON THE MIXING TIME AND LOAF VOLUME OF GLUTEN-STARCH DOUGHS (TURKEY GLUTEN)

Additions to Gluten-Starch Doughs	Absorption	Average Mixing Time	Loaf Volume
	%	*min.*	*cc.*
None	61	19.0	157
Crude albumin	61	9.5	196
Purified albumin	61	20.0	184
Dialyzables	57	18.5	170
Dialyzables + purified pentosans	61	11.0	164
Sucrose	57	16.5	176
Sodium chloride	61	22.0	166
Sucrose + sodium chloride	57	17.5	190

but the rate of gas production during proof in doughs containing 10% sucrose was found to be virtually the same as in doughs with only 6% sucrose, providing 0.25% ammonium chloride was also added. Therefore, the increase in loaf volume caused by extra sugar seemed to be independent of changes in gas production. The extra salt added to doughs raised the salt content from 1.5 to about 1.8% of the weight of flour used.

After the demonstration of the importance of the albumin fraction to baking characteristics, it became of interest to examine other proteins for their effectiveness in supplementing gluten. Baking results with proteins both from wheat and other sources are shown in Table VII. The volume response to the flour albumin preparation was definitely larger than those to the other proteins used.

Discussion

An interpretation of results such as those presented in Table III is complicated by the fact that baking results with replicate fractionations of a single flour varied to some extent. This variation arose

partly from differences in the amounts of soluble proteins retained by the glutens. Such differences affected not only the loaf volumes of gluten-starch doughs, particularly, but also the effectiveness of the total solubles of one flour when added to the gluten of a second flour. Some of the variation among total solubles and albumin fractions was due to the easy alteration of albumin during isolation, shown by partial loss of solubility of the fractions. Alteration of albumin may also be

TABLE VII

THE EFFECTIVENESS OF OTHER PROTEINS AS REPLACEMENT FOR CRUDE WHEAT ALBUMIN IN GLUTEN-STARCH DOUGHS (THATCHER GLUTEN)

Protein Added[1]	*Loaf Volume*	*Volume Response*
	cc.	*cc.*
None	157	
Crude flour albumin	186	29
Gliadin	166	9
Gluten	169	12
Zein	161	4
Com. soybean protein	161	4
Com. egg albumin	166	9
Crude flour globulin[2]	156	−1

[1] Doughs were reconstituted to correspond to the use of flour containing 12% protein, approximately 11% of which was furnished by gluten.
[2] A crude preparation obtained by dialysis of sodium chloride extracts of flour.

responsible for the occasional failure of completely reconstituted doughs to give loaf volumes equal to those obtained with unfractionated flour doughs.

An observation related to this method of detecting alteration during fractionation, but as yet not further investigated, is of interest. Loaf volumes of fully reconstituted doughs equalled those of the original flours if the glutens had not been exhaustively washed and still retained some material which would be considered buffer soluble if more thorough washing had been used. When a more thorough washing was used, and the solubles were treated by the same procedures found adequate to avoid alteration in the first case, it was impossible, at least by our regular procedure, to obtain the loaf volumes that were obtained when less thorough separations were made. Perhaps the solubles less easily removed from gluten were more easily altered, but an alternative possibility is that once the solubles in gluten were thoroughly washed out there was a physical difficulty encountered in trying to work the solubles back into the gluten. The latter explanation appears to be supported by observations of Baker, Parker, and Mize (1) that the elasticity and flow properties of gluten could be modified by kneading in dough wash-water when the gluten had not been thoroughly washed, but no effect was observed on thoroughly washed gluten following similar treatment.

Considerations such as those just discussed indicate that it would

be of considerable advantage in work of this type to have available homogeneous components and adequate methods for estimating the amounts of such components present in different flours. However, the consistent positive responses of gluten-starch doughs to inclusion of solubles contrasts with the variable effects recorded in the literature. This may be a reflection of the use of phosphate buffer rather than water for the separation of gluten and solubles. As is well known, the physical characteristics and yields of gluten may be influenced appreciably by the salt content of the wash water; and it was with regard to these features of the separation that Dill and Alsberg (3) recommended the use of 0.1%, pH 6.8 phosphate buffer. With the five flours used in this work, water and the buffer were found to extract different amounts of nitrogen; furthermore, the water extracts contained relatively more of gliadin-like components as indicated by a higher ratio of amide to total nitrogen. On the basis of this difference in composition the water solubles more closely resembled gluten and might be expected to modify gluten to a lesser extent. A direct comparison of water and buffer solubles of the same flours, both with regard to composition and baking effects, is needed before this explanation can be accepted.

The finding that sucrose or sucrose and salt simulated so well the effects obtained with the dialyzable fraction of flour was unexpected. Micka and Child (9) have observed that a decrease in the absorption of a dough is required as the sucrose content of the formula is increased. Also, Larmour and Brockington (8) observed with one flour that loaf volume increased as the sugar content of the formula was raised even though the respective doughs showed practically equal total gas productions during the proof period and equal rates of gas production as the doughs went into the oven; this result was not observed with two other flours. It seems quite probable, therefore, that the sugars and salts content of the dialyzable fractions could be largely responsible for the mixing and baking responses to this fraction.

An important aspect that is suggested by this study concerns the ability of only 10 or 12% of the total flour nitrogen to modify the behavior of the whole gluten complex. Soluble components, such as albumin, may play an important part in determining the effects of oxidizing agents, mixing, and other treatments which modify dough properties, and, accordingly, may merit more attention than they have received in the past.

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LABORATORY STEEPING PROCEDURES USED IN A WET MILLING RESEARCH PROGRAM [1,2]

S. A. WATSON, C. B. WILLIAMS and R. D. WAKELY

ABSTRACT

Equipment and methods employed in a corn and grain sorghum wet milling research program are described. A new laboratory countercurrent steeping apparatus has been developed which is closely patterned after commercial equipment but which provides for greater control over steeping variables. Steeps making up the batteries are glass jars having a capacity of 400 g. of grain. Water movement across the batteries is brought about by application of vacuum at the draw end. Water movement is initiated by a time clock-solenoid valve system and is stopped by an electrode-relay system. Up to four such batteries can be operated in parallel.

The effect of imposed variables on steeping efficiency is judged by steepwater analyses and by tests on the steeped grain. Determinations made on the steepwater include soluble solids, pH, sulfur dioxide, lactic acid, reducing sugar, ash, and protein. In addition to these familiar determinations a new procedure has been developed by which the relative bacterial activity (R.B.A.) of the *Lactobacillus* species occurring naturally in steepwater can be estimated. The R.B.A. test consists of determining the titratable acid formed when a steepwater sample is incubated for 4 hours at 110°F. in the presence of an excess of dextrose and corn steepwater nutrient adjusted initially to pH 5.0.

Suitability of steeped grain for wet milling is determined quickly by microscopic examination of thin sections of the steeped kernels. The novel feature of this test is a table of standard descriptions with which kernels can be given numerical values for properties related to wet milling. Properties include degree of swelling, pericarp condition, cell wall condition, ease of starch removal and condition of the protein matrix. Final evaluation of the steeped grain is always carried out by a complete wet milling analysis by which the yield and quality of the four wet milling fractions—starch, germ, fiber, and gluten—may be ascertained.

The corn wet milling process has developed over the past 60 to 70 years by a process of evolution (3). Gradual improvement came about largely by trial and error methods, often without a basic understanding of the variables involved and with no fundamental standards by which to evaluate the results.

Steeping is essentially a process of soaking dry grain to soften it for subsequent grinding and starch liberation. The apparent basic vari-

[1] Manuscript received June 19, 1950. Presented at the 1950 annual meeting of the American Association of Cereal Chemists under the title "Starch Quality as a Function of Steeping."

[2] Contribution from the George M. Moffett Research Laboratories, Corn Products Refining Company, Argo, Illinois.

ables in steeping are: (1) the grain, (2) water, (3) time, (4) temperature, (5) lactic acid, and (6) sulfur dioxide.

Water absorption is, of course, essential but appears to represent only a preliminary step. Thus, a maximum moisture content of 42 to 45% is reached in the first 8 to 10 hours, yet the time required for thorough steeping lies between 40 and 50 hours. Moreover, corn steeped in water devoid of either lactic acid or sulfur dioxide, is not suitable for commercial processing.

Soluble substances, located predominantly in the germ, are leached during steeping. This loss of solubles lowers the density of the oil-bearing germ and thereby facilitates subsequent germ separation by flotation.

An integral part of the present steeping system is the maintenance of homofermentative *Lactobacillus* species in the steeps. These bacteria, which convert the soluble sugars from the grain to lactic acid, predominate in the steeps because, unlike other grain-borne microorganisms, they are able to grow at the steeping temperatures of 120 to 125°F. and pH values in the range of 3.8 to 4.4. Subsequent recovery of solids by evaporation of the steepwater is aided by the reduction in pH level resulting from the formation of lactic acid. This acid plays an additional role in steeping by virtue of what appears to be a specific softening effect on the kernel.

In addition to its aseptic properties, sulfur dioxide apparently has a specific effect in loosening the protein matrix in which starch granules are embedded.

Other factors of importance to commercial steeping practice are associated with the peculiarities of a countercurrent battery. Here, water of low solubles content, containing 0.1 to 0.2% sulfur dioxide, enters the system on the oldest grain and is slowly moved from steep to steep. It is finally removed from the system over new grain, being now practically devoid of sulfur dioxide and carrying 3 to 8% of solubles. The volume of water withdrawn in relation to the quantity of grain per steep is spoken of as the draw rate. It affects the extent of solubles removed from the grain, the extent to which sulfur dioxide is carried through the battery, and hence the completeness of the fermentation process. Bacterial activity begins at that point in the battery where the sulfur dioxide level reaches about 0.04%. Therefore, the draw rate and sulfur dioxide concentration in the steeping medium are generally regulated so as to divide the battery into two approximately equal portions; namely, the portion in which sulfur dioxide predominates and the portion where the formation of lactic acid takes place. Other variables include battery length, methods of

advancing water (continuous or batchwise), and advance interval or grind rate.

Attempts to carry out controlled steeping experiments on a full plant or even large pilot plant scale are difficult mechanically and often yield only inconclusive results because of inescapable fluctuations, such as the condition and composition of the grain and composition of mill house waters so that real control is virtually impossible. These and other considerations led to the development of laboratory scale steeping equipment which could be operated with strict control over the numerous variables discussed above. The purpose of this paper is to describe the steeping equipment and the methods employed in determining steeping efficiency.

Experimental Equipment and Technique

Equipment. The countercurrent steeping equipment now used in this laboratory is pictured in Fig. 1. Steeps making up the batteries are one-quart fruit jars each holding up to 400 g. of grain and fitted with a rubber stopper containing inlet and outlet tubes. Each of the twelve stainless steel water baths, in which the jars of grain are im-

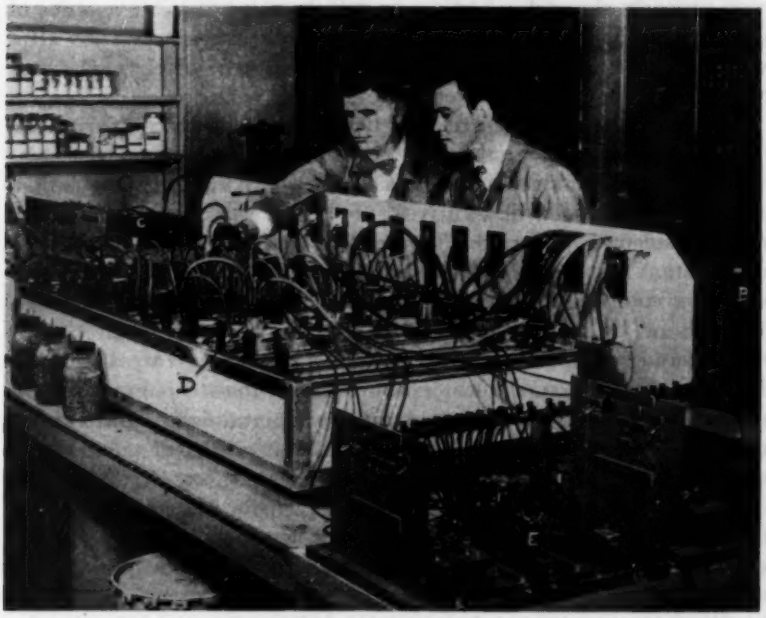

FIG. 1. Laboratory countercurrent steeping batteries and automatic draw apparatus. (A) Supply of steeping medium. (B) Time clock. (C) Solenoid valves on vacuum line. (D) Steepwater draw flasks. (E) Steeping relay.

mersed, has individual temperature control. Thus any combination of temperatures in the same or in different batteries may be compared.

The steeping medium under test enters the system from the supply bottle (A), is delivered to the bottom of the first steep, moves up through the grain, overflows into the bottom of the next steep, and so proceeds across the battery. Advance of water through the battery is accomplished by application of vacuum at the draw end. For this reason each battery must be a closed, liquid sealed unit. At the time the draw is to be made, a time clock (B), through a vacuum tube relay, actuates a solenoid (C) which thereupon opens the vacuum line. Steepwater is pulled from the end steep onto a new steep of dry grain and into the draw flask (D). The vacuum line is closed and the draw stopped when the electrodes in the top of the draw flask are contacted by the rising steepwater. The steepwater, thus drawn, is now available for analysis and the steeped grain is removed from the other end of the battery.

The time clocks (one for each of two batteries) can be adjusted to make draws at set intervals of 2 to 24 hours. This apparatus can also carry out as many as ten consecutive advances and draws at predetermined intervals without manual assistance after the battery has been properly charged. The device which makes possible a series of such operations is the relay (E), which moves to a new position after each draw, thereby cutting out the preceding pair of electrodes and cutting in the next pair in preparation for the next draw.

Although the countercurrent battery is excellent for comparing the effect of operational variables, it is less ideal for studying basic biochemical factors in steeping such as solubles exchange, enzyme action, and the role of chemical additives. For such work, experimentation in single steeps is preferred. Grain of an individual steep, immersed in a constant temperature water bath, may be contacted with a medium of known composition, circulated by an individual pump either continuously or, by use of a timer, at predetermined intervals.

Steepwater Composition as a Criterion of Steeping. In plant operations the composition of the steepwater and of the steeping medium is used almost exclusively as control tests for maintaining uniform steeping conditions. Experience has dictated the ranges of composition which have given optimum milling results.

Each lot of steepwater drawn is analyzed for acidity, pH, sulfur dioxide, and dry substance. Occasional "steeping curves" are determined by these same analyses on water from each steep across the battery. The results are used to manipulate the draw rate, sulfur dioxide concentration, and temperature in order to maintain the steepwater draw at 3 to 5 degrees Baumé, 3.9 to 4.1 pH, titratable

acidity corresponding to 1.0 to 1.5% lactic acid, and 0.01–.02% sulfur dioxide. An obvious objective is the attainment of maximum fermentation of reducing sugars to lactic acid.

Steepwater condition in the laboratory batteries is judged by the tests outlined for plant operation in addition to others which give more complete and accurate data. Since titratable acidity may depend on the types of acids and buffers in the steepwater, lactic acid is measured daily by the permanganate oxidation method (2). Reducing sugar (4) is determined daily as a check on the completeness of the fermentation. Analyses for nitrogen, acetic acid, and ash contents of the steepwater are made occasionally. A typical "steeping curve" on a grain sorghum battery is shown in Table I.

TABLE I

COMPOSITION OF STEEPWATER IN SUCCESSIVE STEEPS ACROSS A LABORATORY COUNTERCURRENT BATTERY OF MARTIN GRAIN SORGHUM

Steepwater Hours in Battery	SO_2	pH	Dry Subst.	Acidity as Lactic Acid[2]	"True" Lactic Acid	Reducing Sugar	Total Protein[3]	Relative Bacterial Activity
	%		%	%	%	%	%	
0[1]	0.10	3.10	1.02	0.50	0.20	0.031	0.38	
8	.078	3.55	1.25	0.45	.21	.067	.45	20
16	.069	3.90	1.65	0.50	.25	.075	.68	20
24	.060	4.05	1.90	0.65	.40	.088	1.00	20
32	.052	4.10	2.01	0.67	.54	.139	1.30	31
40	.039	4.25	3.24	1.00	.70	.114	1.66	72
48	.026	4.30	4.00	1.19	.93	.104	1.96	230
56	.021	4.40	5.17	1.39	1.17	.100	2.50	545
64	0.017	4.45	6.25	1.60	1.52	0.112	2.74	500

[1] Diluted steepwater plus SO_2 to given composition.
[2] Not corrected for acidity of SO_2.
[3] N × 6.25.

Measurement of the population of lactic acid bacteria in steepwater is difficult by the usual bacteriological techniques. A test has been devised in this laboratory by which the acid forming bacterial activity in steepwater can be determined in 4 hours. The method, which is the reverse of a bioassay of a growth factor, consists of short incubation of the sample in the presence of an excess of dextrose and concentrated corn steepwater as nutrients. The culture is adjusted to pH 5.0 and the acid formed during 4 hours at 110°F. (43.3°C.) is titrated to pH 5.0 with standard alkali. The results, expressed as mg. of lactic acid formed per 100 ml. of original steepwater, are designated as Relative Bacterial Activity (R.B.A.). The method assumes that the initial rate of fermentation is proportional to the number and activity of bacteria present. Results are admittedly empirical but values are highly reproducible and are, of course, comparable for a given system. The R.B.A. test has been of considerable value in

following the immediate effect of steeping conditions on the lactic fermentation in the steeps.

Grain Milling as a Criterion of Steeping. A most difficult aspect of the research program has been the measurement of the effect of steeping conditions on the milling properties of the steeped grain. Experimental evaluation of these properties is difficult for several reasons.

a) Satisfactory steeping is defined by standards of milling and separations developed from manufacturing experience. Standards are usually expressed in negative rather than in positive terms.
b) A kernel is an organism, the different parts of which may require different conditions for optimum steeping.
c) The difference between the original kernel and the steeped kernel is one of degree, and a quantitative measurement of the extent of the change undergone is difficult to obtain with reliability. This is true especially of small differences near the optimum of steeping quality.

Since the primary objective of steeping is maximum recovery of starch of highest quality, one form of laboratory evaluation is a complete wet-milling procedure patterned as closely as possible to accepted manufacturing practice. The procedure employed in this laboratory to determine the yield and quality of the four major wet milling fractions—germ, fiber, starch and gluten—will be described.

The first step, degermination, is carried out in a Waring blendor having blunt blades and operated at reduced speed. Equal volumes of steeped corn and water are ground for 1 minute (1½ minutes for grain sorghum). Under these conditions, well steeped grain can be de-

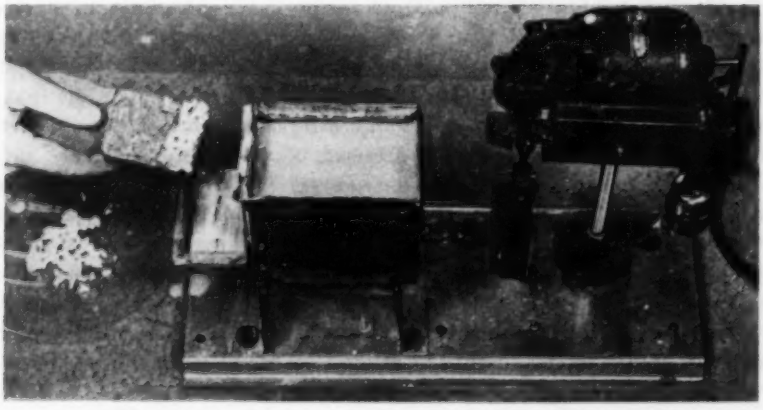

FIG. 2. Laboratory germ separation unit being used for corn germ removal.

FIG. 3. Quaker City mill used to similate the Buhr stone mill. (A) Notched wheel. (B) Counter weights for maintaining constant load on wheel and grinding surfaces.

germinated satisfactorily with a minimum of germ fragmentation. With poorly steeped grain, the number of undegerminated kernels can be used as a measure of steeping quality.

Slurry from the degermination grind is placed in the germ flotation unit shown in Fig. 2. This unit has a slowly rotating paddle in the bottom which keeps the starch suspended thus permitting the lighter germ to rise. The floated germ is repeatedly skimmed by hand using a wire screen until removal is complete. The germ is washed, dried and its yield and oil content are determined.

The second step involves separation of fiber. Residue from the germ separator is poured onto a nylon bolting cloth (openings approximately $61\mu \times 71\mu$) on a shaker and the bulk of the starch-protein suspension is removed from the coarser fraction. Coarse material is transferred to a Quaker City laboratory mill and ground with a small amount of water (Fig. 3). This operation is analogous to grinding with a Buhr stone mill. The spacing between the plates is kept con-

stant by means of a notched wheel (A) which is maintained at constant tension by a weighted pulley arrangement (B).

The discharge from the Quaker mill is returned to the shaker and washed with several liters of water; the fraction remaining on the silk is the fiber fraction. Yield, and starch and protein contents are determined.

FIG. 4. Starch table. Operator is washing residual protein from surface of starch cake.

The fraction which passes through the silk (mill starch) may be put over a 300 mesh screen to determine the amount of ultra-fine fiber and grits which would be retained with the starch in manufacturing operations. A minimum of very fine fiber in mill starch is desirable.

The third step is the separation of starch from the gluten. The mill starch slurry is adjusted to 8° Baumé and passed slowly down a

channel iron "table" 19 ft. long and 2 to 4 in. wide (Fig. 4) which has a mechanism for pitch adjustment.

A pitch of 1 in. per 10 ft. is generally used. The surface of the starch deposited on the table is washed with water and these tailings are combined with the gluten overflow. The starch is reslurried, washed, and filtered. Yield, and protein and starch contents of the gluten, and yield and protein content of the starch are determined. Typical wet milling results are given in Table II.

TABLE II

TYPICAL RESULTS OF FRACTIONATION BY LABORATORY WET MILLING OF STEEPED CORN AND GRAIN SORGHUM

Fraction	Yield d.b	Oil d.b.	Starch		Protein d.b.
			d.b.	Recovery	
	%	%	%	%	%
Corn		5.2[2]	70.5		10.5
Germ	7.2	52.6			
Fiber	8.4		12.1	1.4	10.8
Gluten	11.5		48.7	8.0	39.5
Starch	62.8		99[1]	88.2	0.36
Solubles	7.5			—	
Recovery	97.4			97.6	
Grain Sorghum		3.8[2]	72.0		11.6
Germ	4.3	43.6			
Fiber	7.1		29.0	2.9	19.2
Gluten	11.4		35.9	5.7	40.2
Starch	65.5		99[1]	89.9	0.60
Solubles	7.4				
Recovery	95.7			98.5	

[1] Estimated.
[2] Prior to steeping.

Grain Examination as a Steeping Criterion. The method which has proved to be most valuable in experimental work is examination of thin slices of steeped kernels by a technique similar to that first reported by Cox, MacMasters, and Hilbert (1). This technique has been broadened and standardized so that a grain kernel can be rated numerically for millability. From the examination of many kernels of different degrees of steeping, standard descriptions have been drawn as given in Table III. Five different classifications are used for determining the properties of steeped corn kernels while three of these are used for steeped grain sorghum kernels. Each descriptive classification is divided into several sub-classes, each of which is given a numerical rating using a score of 100 to represent what is thought to be the optimum condition.

TABLE III

DESCRIPTIVE CLASSIFICATIONS FOR DETERMINING STEEPING SCORES BY EXAMINATION OF STEEPED KERNELS

Classification	Score as % of Most Desirable Condition for Corn
For both corn and grain sorghum	
I. Condition of endosperm cell wall network	
a. Very brittle. Endosperm breaks up during sectioning due to adhering starch and protein.	0
b. Whole sections can easily be obtained but on repeated brushing endosperm may break into pieces.	50
c. Strong and resilient when brushed repeatedly.	100
d. Very fragile due to weakened cell walls. Endosperm cell walls largely disintegrate when placed in water.	150
II. Ease of starch removal	
a. Endosperm brittle. Starch firmly retained by protein matrix, cf. Ia.	0
b. Attempt at brushing section breaks up endosperm.	25
c. Starch easily brushed from most of floury endosperm in water, but difficult to brush from horny endosperm even after chloral hydrate treatment.	50
d. Starch drops out of floury endosperm in water. Easily brushed from horny endosperm after chloral hydrate treatment.	75
e. Almost all starch drops out of kernel in water. Horny endosperm largely brushed out in water.	100
III. Condition of protein matrix	
a. Protein matrix strong and intact throughout and starch not released, cf. Ia.	0
b. Protein matrix intact in horny endosperm. Floury endosperm breaks out on sectioning.	25
c. Protein matrix collapsed in many floury endosperm cells, intact in horny endosperm cells.	50
d. Protein matrix entirely collapsed in floury endosperm cells. Outer horny endosperm still largely intact.	75
e. All floury endosperm cells bare of protein. Matrix collapsed in most horny endosperm cells.	100
For corn only	
IV. Dent score	
a. Dent intact.	0
b. Dent partially swollen.	50
c. Dent completely swollen and turgid.	100
V. Pericarp score	
a. Pericarp very tough and wiry. Curls inward when cut.	0
b. Pericarp tough and resilient.	50
c. Pericarp soft and pliable.	100

Longitudinal slices of 40 micron thickness are made from the kernel on a Spencer table microtome with a freezing stage. At the time of sectioning, observations are made of the behavior of the pericarp and

the fragility of the endosperm cell wall tissue. When the section is placed in water the starch may brush out either directly or after treatment with 50% chloral hydrate solution, as used by Wagoner (5) or with the 70% sulfuric acid solution of Cox *et al.* (1), but the use of the chloral hydrate reagent is preferred in this test. Thereafter, an unbrushed section, a section brushed in water alone, and one brushed in chloral hydrate solution are all mounted on a slide, stained with iodine and examined microscopically to determine the extent of starch retention by the horny endosperm. The protein matrix is examined for the properties described in Table III and scored accordingly. Figure 5 shows an enlargement of a portion of a steeped grain sorghum kernel which has been brushed in water. This kernel was given a starch removal score of 80 and a protein score of 60.

The endosperm cell wall network[3] is examined for tears and scored. Observation of many samples of steeped grain indicate that best milling is obtained when the cell wall network is fairly strong. Undue fragility of the cell walls, on the other hand, is believed to be indicative of a condition which results in a large amount of very fine fiber that passes the nylon screens. Accordingly, if the sections break up easily in water they are judged to be oversteeped and are scored above 100%. Three to five individual kernels are usually examined and scores for each test averaged for the sample.

Discussion

Steeping experiments with corn and grain sorghum using the laboratory countercurrent battery have produced results similar to those obtained in commercial steeping as judged by both steepwater composition and steeped grain properties. Only a few of the possible experimental conditions which may be studied with this equipment have been carried out. Some of the experiments that have been conducted include: (1) effect of temperature on lactic acid formation in the steeps, (2) effects of several steepwater draw rates, (3) effects of several sulfur dioxide concentrations, (4) effect of different conditions of drying of grain sorghum, and (5) effect of week-end shutdown on starch viscosity and on the formation of lactic acid.

The size of laboratory steeps has been frequently criticized as being too small in comparison with commercial steeps which carry 2,000 to 3,500 bu. of grain. Such critics do not take into account that the basic unit in steeping is not the steep but the kernel. In 350 g. of corn there are about 1,200 kernels while in the same amount of grain sorghum there are about 12,000 kernels. This is considered to be an adequate sampling of the kernel population. On the other hand, re-

[3] Not to be confused with the protein matrix.

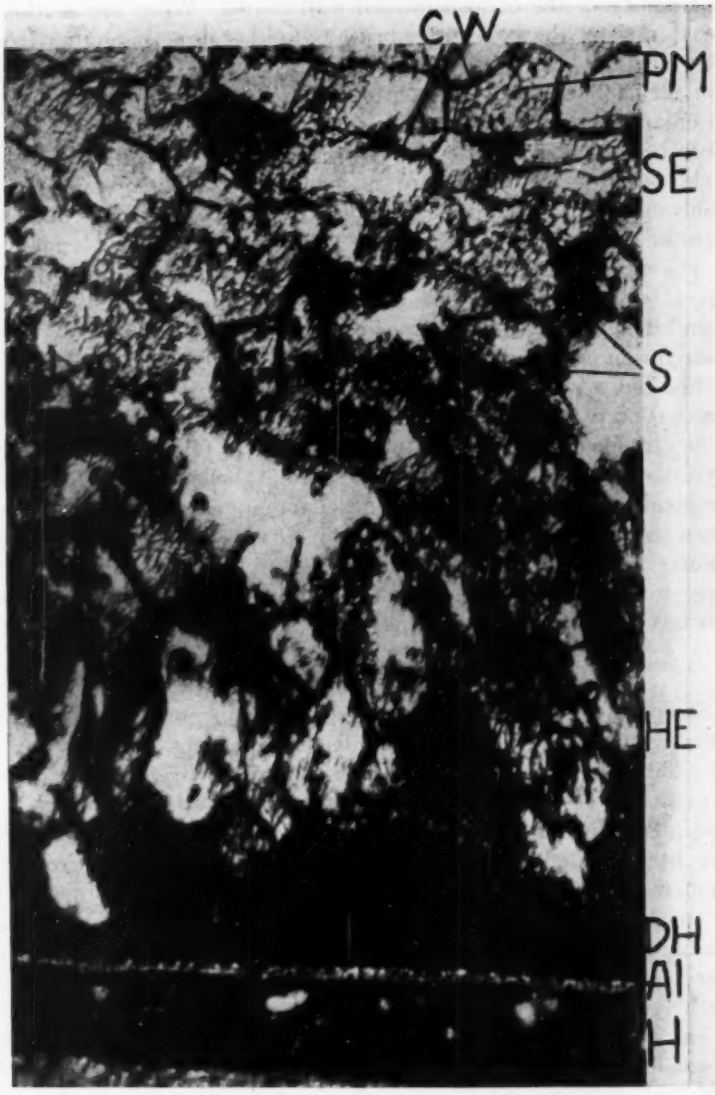

FIG. 5. Section of steeped grain sorghum kernel. 250×. H, hull; A, aleurone; DH, dense horny endosperm; HE, horny endosperm; SE, soft endosperm; PM, protein matrix with starch removed; S, starch granules; CW, endosperm cell walls.

moval of as little as 25 ml. of liquid from each steep for analysis will upset the equilibrium across the battery Samples are taken for steeping curves at the end of an experimental run or if taken during a run, at least 24 hours is allowed for equilibrium to again become established.

Data obtained by the wet milling procedure are essential in giving a final appraisal of steeping in order that the effect of steeping conditions on the recovery and quality of starch can be known. However, because of the large number of operations involved, small differences in steeping are often concealed by cumulative procedural errors. The method is time-consuming and final results are obtained only after considerable analytical work. Therefore, more rapid methods indicative of what may be expected in wet milling are more useful in following the results of steeping experiments.

The most useful method in laboratory studies has been the microscopic steeping score Using this method the condition of the grain can be ascertained in a few minutes after the completion of the experiment. During the course of an experiment a few kernels may be removed from a steep for examination without appreciably altering the grain-liquid ratio The expression of results on a numerical percentage basis facilitates the recording and communication of data. The numbers obtained are admittedly relative and subject to variation in judgment between operators Nevertheless, in the hands of experienced observers, results are surprisingly reproducible and steeping scores among kernels of the same sample are remarkably uniform. Reproducibility of averaged values has been estimated to be about $\pm 2.5\%$.

The scoring and standard descriptions (Table III) are based on yellow dent corn as 100%. This same table can be used for scoring other varieties of corn and grain sorghum but when the ideal conditions are known they may be above or below the optimum for yellow dent corn. Thus the scores for well steeped grain sorghum are usually about 90 because this grain has a greater amount of horny endosperm. Although this test is not suitable as a routine test for manufacturing operations it has value in determining if poor results in mill house operation are due to faulty steeping.

Other rapid methods of grain evaluation which have been used to a limited extent are: (1) determination of the amount of mill starch passing the silk screen as produced by the standard degermination grind, (2) the "break test" in which the volume of starch and protein layers formed when a given volume of 7° Baumé mill starch is allowed to stand for 30 minutes is taken as a measure of the ease of separation of starch from protein, and (3) the weight of residue obtained by passing the mill starch slurry over a 300 mesh screen.

It has been seen that many of the manipulative problems in commercial steeping are concerned with obtaining maximum and sustained growth of heterofermentative *Lactobacillus* species in the steeps. For this reason, the Relative Bacterial Activity test described in this publication appears to have considerable promise as a valuable routine

method for plant operations. The method is simple, rapid and has been found to be quite sensitive to changes in bacterial environment in the steeps.

Cox, MacMasters, and Hilbert (1) showed that corn could be adequately steeped when immersed in a medium containing sulfur dioxide and synthetic lactic acid. This finding has been verified for corn and extended to grain sorghum in our laboratory. It is, therefore, concluded that the fermentation itself is not an essential part of the steeping process except insofar as it is a convenient means of simultaneously producing lactic acid, treating the grain with it, and reducing the pH of steepwater prior to evaporation.

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EFFECT OF EMULSIFIERS ON THE ENZYME SUSCEPTIBILITY OF STARCH DURING STALING OF BREAD[1]

Frances E. Volz[2] and P. E. Ramstad[3]

ABSTRACT

Enzyme susceptibility of starch in bread crumb may be determined by digestion with takadiastase. Marked decreases in enzyme susceptibility occur as bread stales. Addition of polyoxyethylene monostearate or fatty acid monoglycerides (at 0.5% of flour wt.) to bread decreased firmness of the crumb, but had no effect upon the initial enzyme susceptibility of the starch one hour after baking or the rate at which this decreased as the bread aged.

The complexity of the bread staling phenomenon is evident from the literature on the subject. Physical characteristics such as firmness, amount of water-extractable solubles, and water absorbing capacity of bread crumb are drastically altered as bread ages at room temperature. It is generally agreed that these alterations are largely

[1] Received for publication August 29, 1950. Contribution from the School of Nutrition, Cornell University, Ithaca, N. Y.
[2] Research Associate.
[3] Associate Professor of Biochemistry.

the consequence of physical changes in the starchy portion of the crumb (4, 7). It is also known that the susceptibility of starch to enzymatic attack is decreased during staling (5, 6, 8).

Certain compounds having emulsifying properties are capable of retarding some of the physical changes which occur during staling. Polyoxyethylene monostearate and fatty acid monoglycerides are examples of this class of compounds. Favor and Johnston (3) reported that polyoxyethylene monostearate has a retarding effect on the rate at which bread crumb loses its softness with age and upon the retrogradation of starch pastes. Carlin (2) accounted for a similar effect of monoglyceride shortenings upon bread crumb on the basis of a reduction in the amount of water-soluble starch which could be extracted from the crumb. He observed that the addition of soluble starch to doughs containing monoglyceride shortenings caused an increase in crumb firmness.

The purpose of the work reported here was to determine the effect of polyoxyethylene monostearate and fatty acid monoglycerides upon the change in enzyme susceptibility of starch in bread crumb during aging. Enzyme susceptibility was measured by the extent of digestion with takadiastase under standardized conditions.

Methods

Bread Preparation. A straight dough procedure was used with 4% sugar, 4% shortening, 4% non-fat milk solids, 2% salt, and 1.3% active dry yeast based on flour. Two types of emulsifiers were used, namely, polyoxyethylene monostearate[4] (POEMS) and a high purity distilled fatty acid monoglyceride prepared from refined cottonseed oil.[5] When these were added at the 0.5% level, an equal amount of shortening was removed from the formula.

Bread Storage. One loaf of each type of bread was sliced soon after removal from the oven, the other was cooled two to three hours before slicing. Five pair of bread slices for enzyme susceptibility studies were wrapped in aluminum foil. The slices for firmness comparisons were placed immediately in plastic sandwich boxes to prevent desiccation and damage due to pressure on the slices.

Enzyme Susceptibility of Starch in Bread Crumb. A twenty gram sample of the bread crumb was stirred with 55 ml. of distilled water using an electric stirrer. A smooth slurry was obtained after two to three minutes of stirring. This method of preparation has been found to be sufficiently mild to avoid damage to swollen starch granules. Three 10 g. portions of each bread slurry were weighed into

[4] G-2145, polyoxyethylene monostearate. Atlas Powder Co., Wilmington, Del.
[5] Myverol-Type 18-85. Distillation Products Industries Div., Eastman Kodak Co., Rocheste New York.

250 ml. Erlenmeyer flasks and diluted with 0.05 *M* sodium acetate buffer, pH 5.2 at 30°C. The flasks were placed at once in a 30°C. water bath and takadiastase[6] dissolved in the buffer was added at 0, 100, and 200 mg. levels. The total volume of buffer added to each flask was 100 ml. The samples were shaken occasionally during a thirty minute digestion period in the water bath. The enzymatic activity was stopped and the proteins precipitated by the addition of

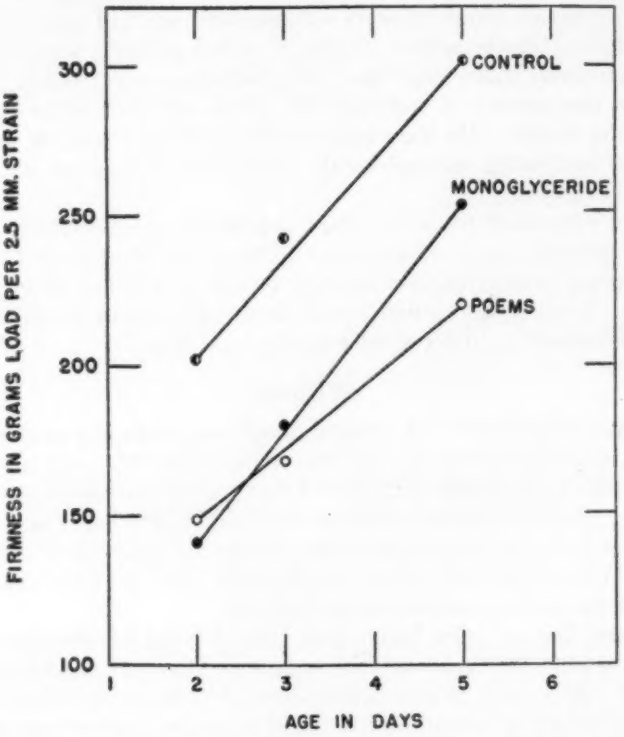

FIG. 1. The effect of added emulsifier (0.5% level) on firmness of bread crumb.

4 ml. of 10% sulphuric acid and 4 ml. of 12% sodium tungstate solution. The digestion mixtures were transferred to 250 ml. volumetric flasks and made up to volume with distilled water. After mixing thoroughly, a portion of the suspension was filtered using No. 4 Whatman filter paper. The first few drops of filtrate were discarded.

Reducing capacity of the filtrates was determined by ferricyanide reduction (1). The reducing capacity of 100 g. dry bread solids cor-

[6] Takadiastase. Parke, Davis and Co., Detroit, Michigan.

rected for enzyme and substrate blanks was considered to be a measure of the susceptibility of the starch to enzymatic breakdown.

Firmness Measurements. The firmness of the breads was determined by means of the Baker compressimeter, according to the method described by Favor and Johnston (3). The crumb firmness was measured on the intact bread slice.

Results and Discussion

The addition of emulsifiers had no discernible effect upon the quality of the bread other than decreasing the "firmness" of the crumb. The differences in firmness depending upon the presence of emulsifiers in the bread and length of time after baking are shown in Fig. 1. The control bread was more firm than that containing emulsifiers. The polyoxyethylene monostearate had a greater effect on firmness than did the monoglyceride.

The starch in bread crumb is highly susceptible to hydrolysis by amylases immediately after baking. This *in vitro* "digestibility" decreases sharply as the bread cools and is held at room temperature. Table I shows the enzyme susceptibilities of bread with and without

TABLE I

THE EFFECT OF 0.5% LEVELS OF EMULSIFIERS ON THE ENZYME SUSCEPTIBILITY OF STARCH IN BREAD

Emulsifier	Takadiastase Level	Reducing Capacity/100 g. Dry Bread Solids[1]				
		Time after Baking				
		1 hr.	4 hr.	10 hr.	24 hr.	72 hr.
	mg.					
None	100	1060	920	720	470	220
Polyoxyethylene monostearate	100	1060	900	720	490	240
Fatty acid monoglyceride	100	970	870	700	460	210
None	200	1220	1060	880	590	280
Polyoxyethylene monostearate	200	1210	1070	910	660	300
Fatty acid monoglyceride	200	1140	1030	880	610	260

[1] Reducing capacity = Milliequivalents of ferricyanide reduced per 100 g. of dry bread solids digested; corrected for the residual reducing capacity of the bread and the enzyme.

emulsifiers at time intervals from 1 to 72 hours after baking. Each value in Table I represents the mean of the results of three bakings.

The enzyme susceptibility of the starch at various time intervals after baking shows a distinct decrease. But, the breads containing emulsifiers do not appear to differ in this respect from the control bread after the first hour. A few enzyme susceptibility determinations carried out sooner than one hour after baking gave lower values for breads

containing emulsifier than for the control breads. The differences, though slight, were consistent but disappeared as the bread cooled.

Starch pastes normally become less susceptible to enzymatic attack as they age, and these studies show that the same change occurs with bread crumb. On the other hand, emulsifiers which altered the firmness of the bread failed to affect the enzyme susceptibility of starch in the crumb after the bread was cool.

Presumably, changes during staling are caused by molecular aggregation of starch. Among other effects, this produces a decreased susceptibility of the starch to enzymatic hydrolysis. It is somewhat surprising, therefore, to find that emulsifiers, which affect the tendency of bread crumb to become firmer during staling, do not perceptibly alter the rate at which enzymatic susceptibility decreases. It is likely that the effect of the emulsifiers upon crumb firmness results from a lubricating effect between the starch granules, while the aggregation of molecules within the partially swollen granules proceeds in the normal manner, causing the decrease in enzyme susceptibility.

It is possible that the *in vitro* enzyme susceptibility as determined here may be correlated with the *in vivo* digestibility of starch in bread. Amylases from animal sources were tried and gave results analogous to those obtained with takadiastase. The latter enzyme was used in the work described here primarily because of its greater stability in aqueous solution.

Acknowledgments

The authors are indebted to Professor Alice M. Briant and Mrs. Rachel Dube of the New York State College of Home Economics who did the baking and to Miss Margaret Bubeck for analytical assistance.

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THE INTERRELATIONSHIPS OF PROTEINS AND AMINO ACIDS IN CORN[1]

KENNETH J. FREY[2]

ABSTRACT

Individual plant samples of the F_2 generation and backcrosses of two maize crosses were analyzed for total protein, zein, tryptophan, valine, leucine, and isoleucine.

Correlation coefficients showed that valine, leucine, and isoleucine were more closely related to one another than to tryptophan in the corn grain. In the samples studied, zein became an increasing proportion of the total protein as the protein percentage increased. Hence, pound for pound, the protein of high protein corn was less nutritionally adequate than that of low protein corn. Tryptophan (an essential amino acid absent in zein) became a decreasing proportion of the total protein as protein percentage increased. Valine became a decreasing, and leucine an increasing proportion of the total protein as protein percentage increased in the corn grain. No trend was apparent for isoleucine.

Weight of corn grain per plant was negatively correlated with protein percentage. Total protein and tryptophan percentages were significantly lower in crossed kernels of corn than in selfed kernels grown on the same ear.

The proteins of corn have been classified as nutritionally inadequate since the turn of the century when it was discovered by Osborne and Clapp (13) that rats died prematurely when corn was the only source of protein in their diet. Numerous other experiments by Osborne and Mendel (14), Willcock and Hopkins (18), Hogan (9), and others, substantiated this discovery. However, Showalter and Carr (15) in 1922 discovered that the proteins of all corn varieties were not identical in composition. They found that protein from high protein corn contained a greater proportion of alcohol soluble protein, zein, than did that of low protein corn. This was important because zein had a very low feeding value. Later, Hansen, Brimhall, and Sprague (6) showed a straight line relationship between total protein and zein; the correlation coefficient was +0.92.

Studies of this nature have considerable academic and practical importance and it was the purpose of the present research to study the interrelationships of certain amino acids in the corn protein.

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[2] Assistant Professor of Farm Crops, Michigan State College, East Lansing, Michigan. Formerly Senior Research Fellow, Farm Crops Subsection, Iowa State College, Ames, Iowa. The author wishes to express his indebtedness to Dr. G. F. Sprague under whose direction this study was made.

Materials and Methods

Materials. The corn samples[3] analyzed in the present study represent material from two sources. Group I included:

1. F_2, Illinois high protein by Illinois low protein.
2. Bc_1, backcross to Illinois high protein.
3. Bc_2, backcross to Illinois low protein.

These two parents were selected because they represented extreme differences in protein percentage. Group I was grown in 1946.

Group II includes:

1. F_2, 1198 × Hy.
2. Bc_1, backcross to 1198.
3. Bc_2, backcross to Hy.

Hy and 1198 are Corn Belt Inbreds. Group II was grown in 1945. Throughout the remainder of this paper the letter designations "F_2," "Bc_1," etc., will be used.

One hundred and fifty kernels of the F_2, Bc_1, and Bc_2, were planted in adjacent 25-plant rows. The plants were selfed to eliminate any effects of foreign pollen, and after accidental losses about 80–110 samples were obtained in each population.

Data obtained on Group I were total protein, zein, tryptophan, valine, leucine, and isoleucine percentages. Data obtained on Group II were total protein, zein, and tryptophan percentages.

The ears used for analysis were well filled with kernels, thus eliminating any modifying effect which poor seed set might have upon protein percentages, such as Hayes (7) found. Only one ear was produced on each plant so that each sample represents all of the grain produced on one plant.

Methods of Chemical Analysis. Each air-dried sample was thoroughly mixed and a two-ounce subsample from each was ground on a Christy-Norris laboratory mill, to pass through a 20-mesh sieve.

Total nitrogen was determined by the Kjeldahl method as described by Willard and Furman (19) and converted to total protein percentage by means of the factor 6.25.

The alcohol soluble protein, zein, was determined by a method slightly modified from that of Hansen *et al.* (6). To 2 g. of corn in a 125 ml. glass stoppered flask was added 50 ml. of 70% ethyl alcohol (sp. gr. 0.865). The flasks were shaken on a mechanical shaker for 30 minutes, allowed to stand over night, and again shaken for 30 minutes. The contents were filtered and 33 ml. aliquots of the filtrate taken for

[3] A sample refers to the grain produced on an individual plant, i.e., an F_1 sample is the corn grain from a selfed ear grown on an F_1 plant. In reality, the protein-producing ability of the genotype of the F_1 plant is being measured.

nitrogen analysis by the Kjeldahl method. One hundred ml. of water were added to the flasks before digestion to prevent foaming.

The method of Sullivan *et al.* (17), modified by Brimhall (unpublished), was used to determine tryptophan percentage of the corn samples. The modifications employed were: (1) the corn samples were peeled before grinding; (2) the p-dimethylaminobenzaldehyde was dissolved directly in the hydrochloric acid; and (3), instead of casein, corn was used as a standard.

The micro-biological assay method of Kuiken *et al.* (10), employing *Lactobacillus arabinosus 17–5*, with modifications by Bollenback (2), was used to determine leucine, isoleucine, and valine percentages in the corn samples.

The modifications were: (1) The medium of McMahan and Snell (12) was used for maintenance of stock cultures. (2) The samples were hydrolyzed with 2 *N* hydrochloric acid for four hours in an autoclave at 15 pounds pressure and sodium hydroxide was used to neutralize the hydrolyzates. (3) The components of the medium were mixed dry and then dissolved. For a discussion of these modifications, see Bollenback (2).

Results

The means and ranges of the percentages of total protein, zein, tryptophan, valine, leucine, and isoleucine for the F_2's and backcrosses in Groups I and II are given in Table I.

One of the purposes of this study was to determine the degree of covariation between the amino acids tryptophan, valine, leucine, and isoleucine in the corn samples analyzed. If these four amino acids are closely related in their variation it would be possible to predict from the analytical data of one of them the percentages of the other three.

TABLE I

MEANS AND RANGES OF TOTAL PROTEIN, ZEIN, TRYPTOPHAN, VALINE, LEUCINE, AND ISOLEUCINE PERCENTAGES FOR EACH F_2 AND BACKCROSS STUDIED

		Protein	Zein	Tryptophan	Valine	Leucine	Isoleucine
		%	%	%	%	%	%
Group I	F_2 mean	9.6	3.3	0.087	0.45	1.42	0.48
	F_2 range	5.9–16.0	1.0–6.7	0.060–0.122	0.27–0.69	0.60–2.66	0.27–0.84
	Bc_1 mean	11.8	4.3	0.098	0.58	1.72	0.66
	Bc_1 range	8.2–18.4	2.1–8.7	0.073–0.118	0.40–0.87	0.78–3.58	0.48–1.04
	Bc_2 mean	6.8	1.9	0.072	0.34	0.88	0.42
	Bc_2 range	4.9–10.2	0.9–3.5	0.057–0.087	0.22–0.54	0.25–1.47	0.27–0.65
Group II	F_2 mean	11.2	3.6	0.106	—	—	—
	F_2 range	8.9–13.3	2.3–5.0	0.087–0.122			
	Bc_1 mean	11.4	3.7	0.110	—	—	—
	Bc_1 range	8.9–13.3	2.3–5.3	0.094–0.122			
	Bc_2 mean	9.9	3.2	0.098			
	Bc_2 range	8.1–11.6	1.8–4.3	0.084–0.115	—	—	—

TABLE II

CORRELATION COEFFICIENTS INVOLVING THE PERCENTAGES OF TRYPTOPHAN, VALINE, LEUCINE, AND ISOLEUCINE IN GROUPS I AND II

Correlation of	Group I			Group II
	F_2	Bc_1	Bc_2	F_2
Tryptophan and valine	+0.56[2]	+0.42[2]	+0.22[1]	+0.21[1]
Tryptophan and leucine	+0.57[2]	+0.41[2]	+0.29[1]	+0.16
Tryptophan and isoleucine	+0.50[2]	+0.40[2]	+0.21[1]	+0.29[1]
Valine and leucine	+0.83[2]	+0.77[2]	+0.67[2]	+0.56[2]
Valine and isoleucine	+0.86[2]	+0.85[2]	+0.60[2]	+0.73[2]
Leucine and isoleucine	+0.72[2]	+0.72[2]	+0.66[2]	+0.86[2]

[1] Significant at the 5% level.
[2] Significant at the 1% level.

The correlation coefficients, given in Table II, between valine, leucine, and isoleucine are quite large, showing that these amino acids are closely related. The relationship between these three amino acids in corn is probably close enough so that analytical determinations for all three are unnecessary in order to be reasonably sure of a high or low content of all three. However, the correlation coefficients between tryptophan and valine, leucine, and isoleucine are somewhat smaller. In fact, only two were greater than 0.5. Probably the tryptophan determination can not be eliminated.

As stated earlier Showalter and Carr (15) found that the protein of Illinois high protein corn contained a larger proportion of zein than that of Illinois low protein corn. When zein is plotted against total protein for the F_2 of Group I, as shown in Fig. 1, the graph shows the same relationship, that as the percentage of total protein in the corn increases, a larger proportion of the protein is zein. All of the other crosses showed the same increasing ratio. In order to test the significance of this increasing proportion of zein it was suggested by Dr. George W. Brown of the Iowa State College Statistical Laboratory that the equation, $y = ax^b$, might be fitted to the data. The utility of this equation lies in the fact that if b is 1.0, the regression line is straight, while if b is greater than 1.0, the line curves upward as in Fig. 1.

To determine if the data of the F_2 of Group I fit the equation, they were plotted on double logarithmic paper as indicated in Fig. 2. Since the above equation can be written

$$\log y = \log a + b \log x,$$

if the graph is described by a straight line when $\log y$ is plotted against $\log x$ then the equation $y = ax^b$, may be taken to represent the data. The graph given in Fig. 2 indicates a linear relationship between $\log y$ and $\log x$.

In Fig. 2 the fitted equation is $\log y = 0.046 + 1.87 \log X$, so that $b = 1.87$. Moreover, applying the usual methods for linear regression, the test of the difference between b and 1.0 resulted in $t = 14.86$, $df = 100$, which is conclusive evidence that the upward curvature in Fig. 1 is characteristic of the population sampled.

The regression coefficients of the logarithms of zein percentage on total protein percentages for each of the F_2's and backcrosses are presented in Table III. Each value of b is significantly greater than unity and is convincing evidence that zein constitutes a larger and larger fraction of the total protein in corn grain of the strains studied as

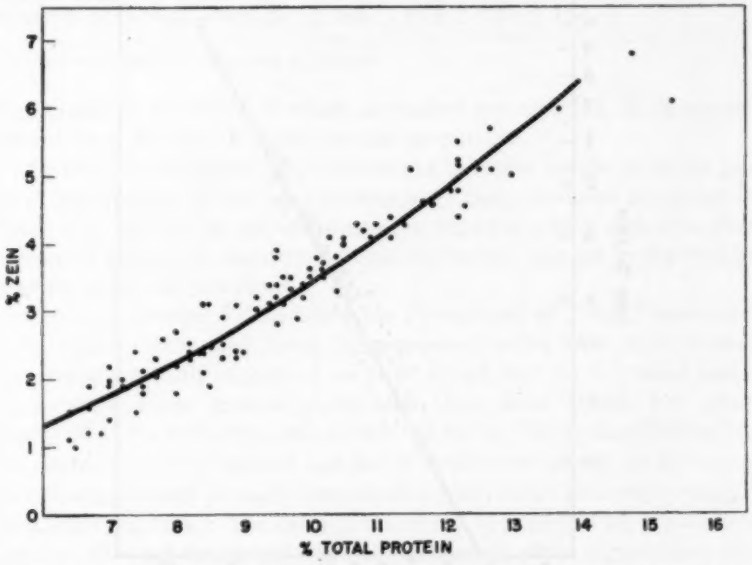

FIG. 1. The regression of zein percentage on total protein percentage in the F_2 of Group I.

the percentage of protein increases. Thus if, as many investigators have shown, zein is the cause of the nutritional unbalance in corn protein it seems safe to conclude that the high protein lines of corn contain a less nutritionally balanced protein than the low protein lines.

Other values of b for the regressions of tryptophan, valine, leucine, and isoleucine on total protein are given in Table III. Each of those involving tryptophan and total protein is significantly less than 1.0. Since tryptophan is one of the limiting amino acids in corn protein this is additional evidence that protein from high protein lines of corn is of poorer quality than that of low protein lines.

The regressions of the logarithms of valine on total protein are all less than 1.0. In Bc_1 of Group I, the regression value is significantly

less than 1.0. This suggests that valine becomes a smaller proportion of the total protein as protein percentage increases in these corn samples. The regressions of log leucine on log total protein were all greater than 1.0, and two of the values were significant. This indicates that leucine becomes an increasing proportion of the total protein as protein percentage increases. Two of the regression values of log isoleucine on log total protein are above 1.0 and two are below. One of the latter is significant, but no trend is apparent for isoleucine. It is of interest to note that in the Bc_1 of Group I, the samples which showed

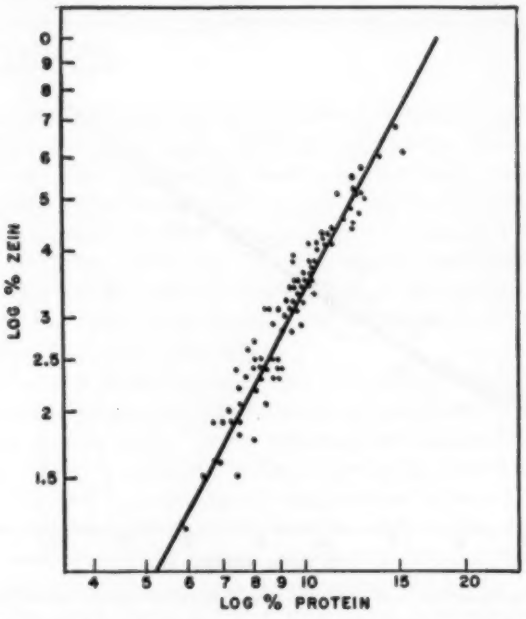

FIG. 2. The regression of log per cent zein on log per cent protein for the F_2 of Group I.

the greatest range in protein percentage and the highest percentage of protein, all had highly significant regression values.

It appears from the regressions of the logarithms of valine and leucine on total protein, that the imbalance noted in the case of tryptophan and zein, is also present with these two amino acids, but to a lesser extent.

Relation Between Total Protein Percentage and Yield of Grain per Plant. East and Jones (3) and Hayes and Garber (8) reported that total protein percentage in corn was negatively correlated with the amount of grain produced per plant. Also it is generally accepted that

TABLE III

REGRESSION COEFFICIENTS OF THE PERCENTAGES OF ZEIN AND AMINO ACIDS ON ON PER CENT OF TOTAL PROTEIN IN CORN GRAIN

Components Involved in Regression Coefficients:	Regression Coefficients, b					
	Group I			Group II		
	F_2	Bc_1	Bc_2	F_2	Bc_1	Bc_2
Zein % on total protein %	1.87[1]	1.56[1]	1.94[1]	1.43[1]	1.74[1]	1.87[1]
Tryptophan % on total protein %	0.37[1]	0.31[1]	0.15[1]	0.27[1]	0.24[1]	0.36[1]
Valine % on total protein %	0.93	0.78[1]	0.86	0.86	—	—
Leucine % on total protein %	1.30[1]	1.42[1]	1.07	1.15	—	—
Isoleucine % on total protein %	1.05	0.74[1]	0.89	1.06	—	—

[1] Significantly different from unity at 1% level.

an increase in the yield of wheat or barley per acre (11, 4) is accompanied by a decrease in total protein percentage.

In the present study the correlations between weight of grain per plant and percent of the total protein were computed and are given in Table IV. All of the correlations were negative which indicates that percent of protein in the corn kernels is inversely related to the weight of grain produced per plant.

Effect of Foreign Pollen Upon the Percentages of Total Protein and Tryptophan. East and Jones (3) compared selfed ears with crossed ears in the same inbred lines of corn and found that those crossed had a significantly lower protein percentage than those selfed, but practically all of the reduction was accounted for by "heterotic dilution" of the protein. When crossed and selfed seeds were grown on the same ear, crossed kernels actually contained slightly more protein by weight than selfed kernels. The same conclusions were drawn by Hayes and Garber (8) who recognized that the maternal plant determined the amount of protein in the grain.

In the present study, paired comparisons were made between selfed and crossed seeds grown upon the same ear. This material was obtained by pollinating Illinois high protein ears (white dent corn)

TABLE IV

CORRELATION COEFFICIENTS OF WEIGHT OF GRAIN PER PLANT WITH TOTAL PROTEIN PERCENTAGE

	Group I			Group II		
Population	F_2	Bc_1	Bc_2	F_2	Bc_1	Bc_2
Correlation coefficient	−0.48[1]	−0.36[1]	−0.22	−0.10	−0.03	−0.27[1]

[1] Exceeds the 1% level of significance.

with a mixture of pollen from the ear-bearing plant and pollen from Krug variety (yellow dent corn). Selfed and crossed seeds were separated according to color. The data from total protein and tryptophan analyses on these samples are given in Table V.

The "t" value for the mean difference of 0.7 in protein percentages between crossed and selfed corn was found to be 2.97, and for mean difference of 0.010 in tryptophan percentage the "t" value was 4.78. Both "t's" are highly significant which indicates that crossing causes a reduction in the percentages of total protein and tryptophan in corn kernels. In order to test for the heterotic dilution of protein in the

TABLE V

TOTAL PROTEIN AND TRYPTOPHAN IN CROSSED AND SELFED KERNELS OF CORN

Sample Number	Total Protein[1]		S-C	Tryptophan %[1]		S-C	Protein per 10 Kernels		S-C	Tryptophan per 10 Kernels		S-C
	Selfed	Crossed		Selfed	Crossed		Selfed	Crossed		Selfed	Crossed	
	%	%					mg.	mg.		mg.	mg.	
1	19.3	18.0	1.3	0.106	0.093	0.013	495	504	− 9	2.72	2.60	+.12
2	18.3	17.8	0.5	0.118	0.106	0.012	372	374	− 2	2.40	2.23	+.17
3	16.2	15.9	0.3	0.118	0.090	0.028	354	372	−18	2.58	2.11	+.47
4	18.3	15.8	2.5	0.094	0.088	0.006	401	366	+35	2.06	2.04	+.02
5	19.0	18.3	0.7	0.104	0.104	0.000	438	455	−17	2.40	2.59	−.19
6	13.8	14.9	−1.1	0.097	0.088	0.009	291	304	−13	2.04	1.80	+.24
7	15.6	15.6	0.0	0.101	0.094	0.007	249	274	−25	1.66	1.65	+.01
8	15.2	13.8	1.4	0.094	0.099	−0.005						
9	13.8	12.7	1.1	0.091	0.086	0.005	295	292	+ 3	1.95	1.97	−.02
10	15.8	15.9	−0.1	0.094	0.086	0.008	343	371	−28	2.04	2.01	+.03
11	17.1	16.2	0.9	0.114	0.102	0.012	413	425	−12	2.75	2.67	+.08
12	19.0	19.3	−0.3	0.104	0.078	0.026						
13	13.0	12.5	0.5	0.110	0.099	0.011	289	291	− 2	2.44	2.31	+.13
14	18.2	16.7	0.5	0.110	0.099	0.011	390	350	+40	2.36	2.08	+.28
15	16.3	14.2	2.1	0.104	0.090	0.014	361	325	+36	2.30	2.06	+.24
Average	16.6	15.9	0.7	0.104	0.094	0.010	361	362	− 1.0	2.28	2.15	+.14

[1] Percentages based upon oven dried material.

crossed seeds, weight of total protein and tryptophan per ten kernels was calculated. In this case, the "t" for the mean difference of 1.0 mg. in weight of protein per ten crossed and ten selfed kernels was −1.53, which is not significant, but the test of significance for a mean difference of 0.14 mg. in tryptophan gave a "t" of 2.63, which is significant. This certainly suggests that the lower percentages of total protein in the crossed seeds is due to heterotic dilution. However, the tryptophan in the crossed kernels was not only lower in percentage, but was actually reduced in amount. No explanation can be given for this lowered tryptophan content.

Discussion

This study indicates that the protein of the low protein corn samples was more nearly balanced nutritionally than that of the high protein

samples. If protein quality was of prime importance, it might be well to select and grow low protein strains of corn. However, the protein content would have to be maintained at a level high enough to meet the amino acid requirements of animals in relation to the amount of energy food consumed.

At least two methods have been suggested for improving the protein quality of the corn kernel. One approach would be to select for larger germ size. This would be effective in two ways: first, germ protein is nutritionally adequate (13), and second, the corn germ contains a higher percentage of total protein than the endosperm. To the best of the author's knowledge, no data have been published which indicate that such a scheme would be effective, but since germ size in corn is readily modified, this approach might be effective. Of course the oil content of corn samples selected for large germ size will likely be increased appreciably because corn germ contains a high percentage of oil.

A second possible method for improving the quality of corn protein would be to select samples with low zein-protein ratio. In a recent paper, Frey *et al.* (5) reported on an experiment of this type in which samples from the segregating population of a cross between strains with a low zein-protein ratio were studied. None of the samples deviated from regression far enough to warrant selection for its low zein-protein ratio. The difficulties involved in the modification of the zein-total protein ratio are further emphasized by data obtained on 61 of the common corn belt inbreds. In only one inbred was a sizeable deviation from regression detected (unpublished data). This suggests that, with the material now being used by corn breeders, it will be difficult to improve the quality of corn protein by selecting for low zein in relation to total protein. Possibly if some corn inbreds were subjected to x-rays, mutant types which had a favorable zein-total protein balance might be produced.

In regard to heterotic dilution of protein percentage, the data in the present study compare favorably with those of East and Jones (3) and Hayes (7). Heterotic dilution is probably due to an increased percentage of carbohydrates which is accompanied by a decrease in the percent of total protein.

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A RAPID METHOD FOR THE DETERMINATION OF POTASSIUM BROMATE IN FLOUR [1]

MARJORIE HOWE [2]

ABSTRACT

A method for the determination of potassium bromate has been developed which involves the oxidation of iodide to iodine by the bromate and titration of the iodine with thiosulfate. The large amounts of interfering organic substances present in flour are separated from the bromate in solution by the addition of zinc sulfate and sodium hydroxide at pH 6.85–6.90. Added bromate can be recovered in amounts as low as 2 p. p. m. The method is applicable when only potassium bromate is present. Other oxidizing substances, such as potassium iodate or potassium persulfate, but not chlorine or chlorine dioxide, interfere with this determination.

There has long been a need for a rapid, quantitative method for the determination of potassium bromate in flour. Many of the methods have been time consuming and have not been adaptable to products

[1] Manuscript received August 30, 1950.
[2] Russell-Miller Milling Company Research Laboratory, Minneapolis, Minnesota.

control. Meyer (6) employed an iodometric titration on a water extract of flour. There are certain substances in the extract which interfere with this titration, necessitating a correction curve. Geddes and Lehberg (3) tried leaching a sample of flour with water, ashing in the presence of potassium hydroxide, oxidizing the bromide to bromine, and titrating with 0.001 *N* sodium thiosulfate. This method is adaptable to the determination of bromate in the presence of chlorates, iodates, and persulfates, but the complexity of the method makes it inadequate as a control procedure. Also, bromide residues from methyl bromide used as a fumigant could interfere in this method. Geddes (2), in his report as associate referee of the Association of Official Agricultural Chemists on the determination of bromate in flour, made use of a carbon tetrachloride flotation procedure to separate the bromate which was then determined iodometrically. In this report a complete method has also been described for the determination of potassium bromate in the presence of potassium persulfate and iodate. This method is again too time consuming for use as a control procedure. Hoffer and Alcock (4) extracted flour with 25% potassium chloride in acetic acid and measured the optical density of the amylose-iodine solution. Johnson and Alcock (5) employed the spectrophotometer with the above technique. Recently Auerbach *et al.* (1) published a method for the determination of persulfate in flour and dough, which involves the re-oxidation of leuco-fluorscein to fluorscein through the action of persulfate ion. Auerbach's technique has been modified and adapted by the present author to the determination of potassium bromate.

Procedure

Reagents. (1) Potassium bromate, 0.01 *N* (0.2784 g. $KBrO_3$ diluted to one liter. Ten ml. of 0.01 *N* stock solution diluted to 100 ml. for 0.001 *N*, which should be made fresh daily). (2) Sodium thiosulfate, 0.01 *N* (2.482 g. $Na_2S_2O_3 \cdot 5H_2O$ and 3.8 g. of borax diluted to one liter. 10 ml. of 0.01 *N* stock solution diluted to 100 ml. for 0.001 *N*, which should be made fresh daily). (3) Ziuc sulfate, 0.18 *N* (51.7 g. per liter). (4) Sodium hydroxide, 0.18 *N* (7.2 g. per liter). (5) Sulfuric acid, 10% (56.5 ml. concentrated H_2SO_4 per liter). (6) Potassium iodide solution, 30% (4 ml. per liter of 2.0 *N* NaOH added as a preservative). (7) Starch solution, 1% (freshly prepared).

Determination. Weigh 10 g. of flour and 0.5 g. of filtercel into a 500 ml. Erlenmeyer flask and add 200 ml. of water. Stopper the flask and mix with a swirling motion for 2 minutes. It is important that the amount of foam be kept at a minimum. Let the mixture stand for 10 to 15 minutes; then add 25 ml. of 0.18 *N* zinc sulfate and

25 ml. of 0.18 *N* sodium hydroxide, swirl, and let stand until the supernatant is clear (about 5–10 minutes). Filter through a fluted, No. 40 Whatman filter paper, discarding the first few drops. To 50 ml. of filtrate, add 10 ml. of 10% sulfuric acid, 3 ml. of 30% potassium iodide, and 1 ml. of starch solution. Titrate with 0.001 *N* sodium thiosulfate. Match the color against a flask containing 50 ml. of water. The blank is obtained by titrating 0.001 *N* potassium bromate with 0.001 *N* sodium thiosulfate. Each 50 ml. aliquot contains the bromate present in 2 g. of flour.

Potassium bromate, p. p. m. =

$$\frac{27.84 \times (\text{titration volume} - \text{blank})}{\text{weight of flour represented in aliquot}}$$

For amounts over 40 p. p. m., use 25 ml. aliquots and 25 ml. of water.

For amounts of 10 p. p. m., or less, or for flour containing coarse particles of potassium bromate, the following adjustments are necessary. Use 40 g. of flour, 150 ml. of water, 50 ml. of 0.18 *N* zinc sulfate,

TABLE I

POTASSIUM BROMATE RECOVERY

Type of Bromate Addition	Bromate Added	Bromate Found	Recovery
	p. p. m.	*p. p. m.*	%
Added to a patent flour in the form of a pre-mix	2	2	100
	4	4	100
	8	8	100
	15	15	100
	23	23	100
	31	32	103
	38	39	103
	46	46	100
	54	53	98
	61	60	98
	69	65	96
	77	78	101
Added in solution to an untreated patent flour	14	14	100
	42	41	98
	56	56	100
	69	71	103
Added in solution to an untreated clear flour	14	14	100
	28	28	100
	42	42	100
	56	55	98
	69	69	100
Added in solution to a clear flour treated with 20 g. of chlorine dioxide	14	14	100
	28	30	107
	42	42	100
	56	57	102
	69	69	100

and 50 ml. of 0.18 *N* sodium hydroxide; proceed as above. Each 50 ml. aliquot contains the bromate present in 8 g. of flour.

Results and Discussion

The results of the addition of varying amounts of potassium bromate to a standard flour are summarized in Table I. The bromate was added to the flour in the form of a premix which was made by mixing finely ground (150 mesh) potassium bromate and wheat starch in a Waring Blendor. Then the premix was added to the flour and mixed for 2 hours. Table I contains the data obtained for the addition of potassium bromate in solution to an untreated patent, to an untreated clear, and to a clear treated with chlorine dioxide.

It should be emphasized that, while the recovery of bromate added to flour can be accomplished with a high degree of accuracy by the method outlined, this method is not highly specific and, if iodate or persulfate are present, they will interfere. From a practical standpoint, however, benzoyl peroxide, chlorine, and chlorine dioxide (the bleaching and maturing agents commonly used on flour) do not interfere and, since iodate and persulfate are not permitted in the Standards of Identity of Wheat Flour, bromate normally is the only compound present that would be measurable by the method outlined.

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FURTHER STUDIES ON THE REACTION OF WHEAT PROTEINS WITH REDUCING CARBOHYDRATES[1]

I. HLYNKA AND J. A. ANDERSON

ABSTRACT

In a study of the carbohydrate-protein interaction, reducing values by the Chapman and McFarlane method were determined on material prepared from high, medium and low protein samples of five varieties of hard red spring wheat grown in Western Canada.

The protein level of flour had an important effect on the reaction; high protein flours gave the lowest initial reducing values and also the greatest increases in reducing value after a storage period of about 6 months. When glucose was added and intimately mixed by adding water, and moisture removed to the original level, reducing values after storage were increased several-fold.

The general picture obtained from analogous experiments with gluten prepared from different wheat varieties of different protein levels was similar to that for flour. However, gluten from low protein flour showed a greater reactivity towards added glucose than gluten from high protein flour.

In the range 20° to 70°C. the temperature coefficient for the gluten-glucose system is approximately 3.6.

The basic importance of the carbohydrate-protein reaction in stored food products may be judged by the large number of studies which can be found on this subject. In view of the importance of chemical changes which take place in flour during storage, it was decided to investigate the carbohydrate-protein interaction between added and endogenous carbohydrates on the one hand and flour protein on the other. A preliminary communication on this work has already been published (5).

The present study on the reaction of glucose with wheat proteins was carried out in the "dry phase" corresponding to a moisture level of about 12% in flour and about 8% in air-dried gluten. In a general way these conditions are similar to those used by Henry, Kon, Lea and White (4) in their extensive study of the deterioration of milk powder and later by Lea (6) and Lea and Hannan (7) in a study of simpler systems of glucose and other sugars with milk proteins and with purified casein. Lea and Hannan also showed that maximum reactivity was at a relative humidity of 65 to 70% which is equivalent to about 13% moisture in flour (1).

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Mohammad, Fraenkel-Conrat and Olcott (11) and Mohammad, Olcott and Fraenkel-Conrat (12) studied the carbohydrate-protein reaction in a solution of bovine serum albumin and other proteins, with glucose and acetaldehyde as the source of carbonyl group. Glucose and acetaldehyde gave much the same results except that the latter was more reactive. These authors showed that under conditions of their experiments there was up to fourfold increase in apparent molecular weight of the reacted bovine serum albumin. They interpreted the results as indicating that glucose and acetaldehyde acted as cross-linking agents.

Several investigators have published temperature coefficients or activation energies for the carbohydrate-protein reaction for different proteins. For the casein-glucose system in the "dry phase" Lea and Hannan (7) found a temperature coefficient of 5.4 between 15° and 25°C. and 3.5 between 60° and 70°C. They give an average activation energy of 29 kcal. Mohammad, Fraenkel-Conrat and Olcott (11) give an activation energy of 30.2 kcal. for bovine serum-glucose system in solution. For the reaction in a variety of egg powders Pearce (13) gives values between 22 and 32 kcal.

The study on the interaction of carbohydrates with wheat proteins has now been extended to include material from five wheat varieites, each at three protein levels, and further information has been obtained about the reaction itself.

Materials and Methods

Material from five wheat varieties was studied. The varieties were Marquis, Thatcher, Redman, Rescue and Red Bobs wheat grown in 1948 at 16 stations in Western Canada. Three protein levels were selected. A medium protein series was obtained by compositing material from all stations for each variety. In addition, a high protein series was obtained by selecting material from two high-protein stations, and a low protein series by selecting material from two low-protein stations. Data summarizing the protein content of flour from these varieties are given in Table I.

TABLE I

PROTEIN CONTENT OF VARIOUS FLOURS (14% MOISTURE BASIS)

Variety	High Protein Series	Medium or Composite Series	Low Protein Series
Marquis	13.9	12.4	11.8
Thatcher	14.8	13.4	11.3
Redman	14.4	13.3	11.2
Rescue	15.4	12.9	10.5
Red Bobs	13.6	11.5	10.6

Experimental samples were then prepared from each of the 15 flours as follows: (1) flour, untreated; (2) flour plus 5% glucose, mechanical mixture; (3) dough from No. 1 air dried and ground to go through No. 40 Wiley sieve; (4) dough from No. 2 air dried and ground; (5) gluten, air dried and ground; (6) gluten plus 5% glucose, mechanical mixture; and (7) gluten plus 5% glucose reconstituted with water to ensure intimate mixing of ingredients, air dried, and reground.

No attempt was made to standardize the moisture content of the samples, but for material of the same kind moisture content did not vary greatly. The average moisture content was 12.7% for flour, 8.3% for dried dough, and 8.9% for dried dough-glucose. The average moisture content of gluten was 7.6% and of gluten-glucose, 9.3%. The samples were stored in tightly sealed glass jars at laboratory temperature.

All the samples were assayed for reducing activity by the Chapman and McFarlane method as described in previous publications (3, 5). An initial assay was made when the samples were 3 to 10 weeks old and a final assay when they were 30 to 39 weeks old, so that the average storage period was about 30 weeks.

Results

The results of the main experiment on the study of reducing values of wheat varieties at different protein levels will be presented first. Table II summarizes data from experiments with flour. The notation "flour + glucose" in the side headings designates a mechanical mixture of flour with glucose, while "dough × glucose" indicates that conditions for interaction were brought about by wetting the ingredients to ensure intimate mixing, and then redrying the material to approximately its original moisture content. In other respects the table is self-explanatory.

Since the results for different varieties are analogous, it will be sufficient to follow the data for any one variety. Flour has a small initial reducing value towards the Chapman and McFarlane test. This may simply be a blank of the experiment. However, other evidence presented in this paper shows that it is likely that a large part of the initial reducing value indicates the presence of a carbohydrate-protein complex in flour.

It is interesting to note that the high protein flour gave the lowest initial reducing value, and that composite (medium protein) flour gave the highest value. This is true for all wheat varieties for material labelled flour, flour + glucose, and dough; the only apparent exception is Marquis flour. These results suggest that the reducing value depends on the amount of reducing carbohydrate available

TABLE II

REDUCING VALUES OF FLOUR-GLUCOSE SYSTEMS (MOLES $K_4Fe(CN)_6$ PER g. $\times 10^{-6}$)

Materials	Low Protein		Medium Protein		High Protein	
	Initial	Final	Initial	Final	Initial	Final
Marquis						
Flour	2.3	2.8	2.7	3.8	2.3	4.4
Flour + glucose	2.0	2.7	2.6	3.9	1.8	4.1
Dough	2.3	3.2	2.3	6.1	2.0	5.4
Dough × glucose	2.8	7.1	3.6	12.0	2.8	11.0
Thatcher						
Flour	2.5	2.9	2.4	3.7	2.0	4.1
Flour + glucose	2.2	2.9	2.4	3.5	1.8	3.9
Dough	2.2	3.1	2.3	6.0	2.0	5.3
Dough × glucose	2.9	7.3	3.3	12.0	2.8	11.0
Redman						
Flour	2.3	2.7	2.6	3.8	1.8	3.8
Flour + glucose	2.0	2.1	2.4	3.7	1.8	3.6
Dough	2.0	3.1	2.2	5.7	2.0	5.1
Dough × glucose	3.2	6.9	2.7	12.0	2.7	10.0
Rescue						
Flour	2.4	2.9	2.5	3.9	1.9	4.0
Flour + glucose	2.1	2.4	2.5	3.6	1.7	3.8
Dough	2.2	2.8	2.0	5.3	1.9	5.8
Dough × glucose	2.3	6.5	2.7	11.0	3.5	11.0
Red Bobs						
Flour	2.5	3.2	2.6	3.9	1.9	4.2
Flour + glucose	2.1	2.6	2.2	3.7	1.8	3.9
Dough	2.4	3.0	2.2	5.4	1.9	5.8
Dough × glucose	3.0	7.0	2.7	11.0	3.5	10.0

rather than on the protein. Some interaction of environment is suggested by the relatively high reducing value of composite samples. In each case, the dough × glucose samples gave higher initial reducing values. This is attributed to a small reaction which takes place during the preparation of the material and the time which lapsed before the initial assay was made. There are individual differences among varieties but no significance can be attached to these at the present time.

An increase in reducing value after a reaction or storage period of about 30 weeks is shown by all samples. Flour shows a small but definite increase; the low protein material shows a much smaller increase than high protein material. Control experiments with a mechanical mixture of flour and glucose give results almost identical with those for flour. However, a second control in which flour was wetted to make a dough, then redried, shows a small increase in reducing values as a result of this treatment. These results indicate an

interaction between the endogenous flour protein and carbohydrates; its extent is apparently associated with protein content. Largest increases in reducing value on storage were obtained in samples in which dough and glucose were intimately mixed by making a dough and redrying (dough × glucose). Again the low protein material showed the smallest increase. Variation among the varieties is small.

A series of experiments analogous to those carried out on flour and dough was also done with gluten prepared from each flour. The data are summarized in Table III.

TABLE III

REDUCING VALUES OF GLUTEN-GLUCOSE SYSTEMS
(MOLES K_4 $Fe(CN)_6$ PER g. × 10^{-6})

Materials	Low Protein		Medium Protein		High Protein	
	Initial	Final	Initial	Final	Initial	Final
Marquis						
Gluten control	7.7	11.1	4.7	11.0	4.9	9.0
Gluten + glucose	6.9	10.8	4.9	11.0	5.1	8.4
Gluten × glucose	32.0	78.0	14.0	62.0	20.0	56.0
Thatcher						
Gluten control	7.2	8.9	4.5	11.0	5.0	8.5
Gluten + glucose	6.8	9.0	4.6	10.0	5.0	8.5
Gluten × glucose	23.0	60.0	14.0	67.0	20.0	44.0
Redman						
Gluten control	7.6	9.2	4.6	10.0	4.6	7.9
Gluten + glucose	6.7	8.9	4.5	11.0	4.9	7.9
Gluten × glucose	22.0	56.0	13.0	67.0	17.0	44.0
Rescue						
Gluten control	6.8	9.4	4.7	10.0	6.1	9.2
Gluten + glucose	6.8	9.3	4.2	11.0	5.7	9.1
Gluten × glucose	24.0	56.0	14.0	66.0	22.0	56.0
Red Bobs						
Gluten control	7.4	11.1	4.9	11.0	6.1	11.0
Gluten + glucose	6.8	10.4	4.5	11.0	5.8	10.0
Gluten × glucose	25.0	59.0	14.0	70.0	20.0	55.0

A very large increase in reducing value of gluten reacted with glucose (gluten × glucose) in comparison with unreacted gluten is immediately apparent. Interesting, too, is the observation that gluten from low protein flour gave a larger initial reducing value but a smaller increase in reducing value on storage in comparison with gluten from medium and high protein flour. These results are similar to those obtained in experiments with flour already described. However, results for low-protein-flour gluten reacted with glucose (gluten × glucose) differed from the results on flour. A larger increase in reducing value was obtained with gluten from low protein flour reacted

with glucose than with gluten from medium or high protein flour similarly treated. The large initial value for gluten × glucose material shows that the gluten-glucose reaction is rapid and that it took place to a considerable extent during the preparation of the material before it was redried and the initial assay made. Some variability in the reactivity of gluten is shown in results for different varieties, but the data are not adequate to draw definite conclusions.

Results of several smaller experiments, designed to obtain further information about the reaction itself, merit consideration. One experiment was designed to provide evidence that reducing activity was indeed associated with the wheat proteins. For this purpose glutens from Thatcher and Red Bobs which had been reacted with glucose in the main experiment were submitted to several hours washing and working in running water until all soluble material had been washed away. These glutens were redried and reducing values determined. Similarly, reducing values were determined on these glutens after they had been extracted with Skellysolve F 95 for 18 hours. The results are shown in Table IV. These data show that there is a small decrease

TABLE IV

REDUCING VALUES ON WASHED AND FAT-EXTRACTED GLUTEN
(MOLES $K_4Fe(CN)_6 \times 10^{-6}$ PER g.)

Treatment	Red Bobs	Thatcher
Untreated		
Before storage	1.4	1.4
After storage	7.0	6.7
Washed	5.9	5.5
Fat extracted	6.4	6.2

in reducing value of glutens reacted with glucose after washing in water and after extracting with a fat solvent. This decrease, however, is only 10 to 15% as compared to a 500% increase which took place in these samples during the reaction or storage period. It is concluded, therefore, that the reducing property which results from the interaction of gluten with glucose is associated with the gluten and that reacted glucose is bound to the gluten.

Information was also sought on the influence of temperature on the wheat protein-carbohydrate reaction. Samples of gluten plus 5% glucose, dough plus 5% glucose, and flour were heated at temperatures from 30° to 70°C. for 18 hours, and the rate of increase in reducing values was determined.

Results are summarized in Fig. 1. In order to show the three materials on the same axes, the ordinate is expressed in terms of percentage of initial reducing value of flour which is taken as 100.

Flour shows no reaction. In fact a slight decrease in reducing value is indicated. Dough glucose shows a progressive increase in reducing value with a rise in temperature. This rate of increase may be characterized by the conventional temperature coefficient for the reaction which is about 2.2 for the range. Gluten-glucose shows a much more rapid rate of increase in reducing value with temperature. The temperature coefficient for this system is approximately 3.6 for the entire temperature range. The difference in the magnitude of the two temperature coefficients may be attributed to the diluting effect of the large amount of starch present in the dough and not in gluten.

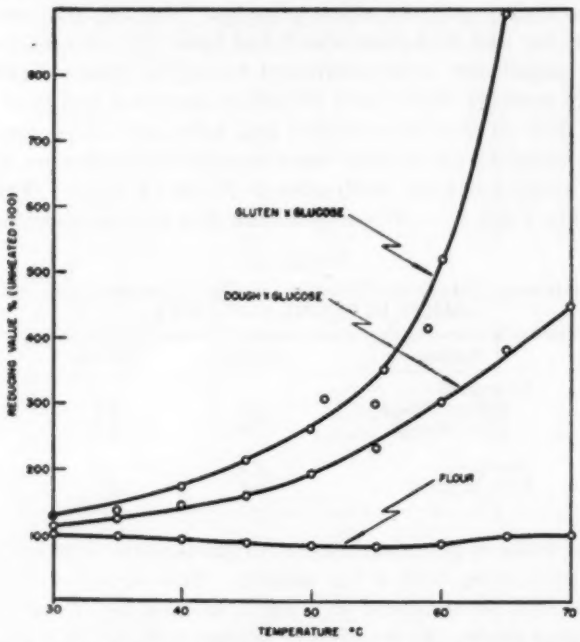

FIG. 1. Influence of temperature on reducing value of flour, dough + glucose and gluten × glucose.

Finally, several experiments which gave essentially negative results are mentioned. Mixtures of gluten with 5% glucose were reconstituted with water and allowed 1, 2, 4, 6, and 18 hours at 30°C. before air-drying the samples. No reaction could be shown with the Chapman and McFarlane test. Similarly formol titrations were carried out on a large number of samples studied in the main series. One gram amounts were titrated using 0.1 *N* sodium hydroxide and 1 ml. of 1% phenolphthalein. No obvious differences were established between the material which was reacted with glucose and unreacted gluten.

Discussion

In general, the results obtained in this investigation confirm our preliminary work (5). The carbohydrate-protein reaction which has been studied in a wide variety of substances has now also been shown to take place in flour and dried dough between wheat proteins and endogneous reducing carbohydrates or added glucose. What flour carbohydrates react has not been established. However, the work of Baker, Parker, and Mize (2) in which they show insolubilization of pentosans or wheat gums with protein in dough through the action of oxidizing agents is suggestive. Moreover, Lewis and Lea (9) established that the pentoses, xylose, and arabinose were the most reactive among the carbohydrates which they studied.

In flour, protein is present in excess in comparison with reducing carbohydrate. It might have therefore been expected from first principles that low protein flour would have a high content of carbohydrate-protein complex. The complex seems to be reasonably stable, since the same picture is carried over to the gluten. The effect of washing does, however, show up; gluten from low protein flour shows a high reactivity to added glucose. A principle analogous with that of mass action seems to govern.

No differences have been established among wheat varieties. It must be pointed out, however, that these varieties are all hard red spring wheat and by and large are very similar in their baking performance. The close similarity of chemical properties is therefore not surprising.

It is interesting to note that the temperature coefficient for the gluten-glucose system is identical with the coefficient Lea and Hannan (7) give for casein-glucose in the range 60° to 70°C., and lies in the lower part of the range of activation energies given by Pearce (13). All these results are on the reaction in the dry phase. Activation energies obtained on liquid phase systems appear to be slightly higher.

A limitation of the Chapman and McFarlane test as a measure of the carbohydrate-protein interaction has been noted. An unsuccessful attempt to demonstrate a reaction between glucose and wet gluten has already been described. Yet the original work of Maillard (10) and numerous other works such as those of Mohammad *et al.* (11, 12) show that the carbohydrate-protein interaction does take place in solution. It is likely that the Chapman and McFarlane test is applicable to reaction in the dry phase but fails in the liquid phase system. A similar limitation is obtained for the formol titration. Such limitations of experimental methods can be readily appreciated if it is kept in mind, as Lea and Hannan (8) point out, that the carbohydrate-protein reaction takes place in several stages with different chemical properties

at each stage. The formation of reducing substances takes place during the first stage and is independent of subsequent brown pigment formation.

On the basis of previous work a hypothesis was proposed (5) that reducing carbohydrates act as cross-linking agents between protein molecules. This hypothesis has been supported, especially by the molecular weight data of Mohammad and his co-workers (11, 12). It is well known that important chemical and physical changes take place in flour on storage and in dough during the bread-making process. The chemistry involved is no doubt complex, but the carbohydrate-protein interaction and the cross-linkage hypothesis appear to be part of the general picture.

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CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF NONFAT MILK SOLIDS. IV. MINIMUM HEAT TREATMENT FOR MAXIMUM LOAF VOLUME[1]

U. S. Ashworth and Gordon J. Krueger[2]

ABSTRACT

Samples of nonfat milk solids experimentally made with carefully controlled preheat treatments were incorporated into a straight dough formula at a level of 8% of the flour. The baking quality of these milk solids tended to be inversely proportional to the amount of undenatured whey protein remaining in the range where 50–90% of the whey protein had been denatured by heat. Maximum loaf volumes were secured when the undenatured whey protein nitrogen amounted to less than 2 mg. per g. of nonfat milk solids, attained by heat treatments of 80°C. for at least 15 minutes. Minimum loaf volumes were found when 15–30% of the whey protein had been denatured by heat. These were about 8.2% lower than the no preheat control volumes (each an average of 20 loaves.)

The fact that the preheating of fluid milk improves the baking quality of the dried product has been established by both laboratory and commercial experience. It is generally assumed by industry that the optimum preheat treatment is gained by subjecting the milk to a temperature of 82°C. (180°F.) for 30 minutes.

Studies at this laboratory (4) and at Minnesota (8) have shown that undenatured whey protein may be responsible for the poor baking quality attributed to unheated milk. A test for baking quality based upon the amount of undenatured whey protein left in the milk powder has been developed (5). Since this test is being rather widely used for the screening of nonfat milk powder manufactured for use in the baking industry, it was thought desirable to find just how closely the denaturation of the whey proteins, as measured by our test, could be correlated with baking quality as measured by loaf volume. For this purpose we prepared several samples of milk powder using our pilot plant spray dryer which is similar to the one described by Coulter (1). With this dryer and a small vacuum pan for evaporating the milk before passing it through the dryer we were able to produce powder without denaturing any of the whey proteins present in the original raw milk. The baking tests were conducted in two independent laboratories.

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[2] Present address: American Can Co., Research Division, Maywood, Ill.

Materials and Methods

A supply of nonfat dry milk solids was prepared without preheat treatment for the first baking tests, so that all heat treatments and baking tests could be made with milk reconstituted from the same batch of powder. It seemed probable that the effect on loaf volume would be the same whether the milk was preheated before drying or whether it was heated after reconstitution and just before incorporating it into the dough. In order to obtain 8% milk solids in the dough formula, without excess liquid, the milk was reconstituted in the ratio of 16 parts solids to 100 parts of water (13.8% total solids) rather than the normal milk concentration of approximately 9% solids. Extensive data have shown (6) that this difference in solids concentration (9 vs. 13.8%) changes only slightly the percentage of whey protein denatured by heat. Moreover, this difference should be of no great significance, since the effect on loaf volume of the different heat treatments was to be compared directly with the amount of whey protein left undenatured as measured by our test for baking quality (5).

Heat treatment of the reconstituted milk samples was conducted in 7 × ⅞ inch, thin walled, loosely stoppered test tubes suspended in a rapidly stirred water bath thermostatically controlled at the desired temperature. Three minutes were allowed for bringing the milk sample to the temperature of the bath. The temperatures were measured by two calibrated thermometers, one in a sample tube and the other in the water bath. After reaching the selected temperature, the samples were held at that temperature for the desired length of time then rapidly cooled to room temperature in cold water, requiring about 2 minutes. Heat treatments were carried on at 70, 75, 77.5, 80, and 85°C. for each time interval of 5, 15, and 30 minutes. When the heat treatment was done before drying, 10 gal. of freshly separated milk were heated to the desired temperature in a steam-jacketed 30 gal. capacity hot-well with continuous mechanical stirring. Ten to 15 minutes were required to attain the final temperature, but less than 5 minutes to return to room temperature.

After heat treatment, the remaining undenatured whey proteins were determined by the turbidity method (5). In this method, the casein and denatured whey proteins were first precipitated quantitatively by saturation with sodium chloride. The filtrate was then acidified to precipitate the undenatured whey proteins and the turbidity compared in a colorimeter with that of a standard. The amount denatured was found by subtracting these values from the corresponding values determined on the reconstituted unheated milk. The baking formula and procedures used by each laboratory are given in Table I.

TABLE I

BAKING INGREDIENTS AND PROCEDURE USED

Baking Laboratory Dough Ingredients	A Grams per mix[1]	B Grams per mix
Flour	200	100
Nonfat dry milk solids	16	6
Sugar	16	6
Yeast (baker's)[2]	5	2
Salt	4	1.5
Shortening	3	3
Water	Optimum amount	Optimum
Malt syrup	0	0.25
K Bromate	0	0.002
Procedure	**Minutes**	**Minutes**
Mixing time (64 rpm.)[3]	4	2.75
Fermentation time (86°F.)	200	105
First punch[4]	120	50
Second punch[4]	175	25
Proofing time (86°F.)	65	55
Baking time (410°F.)	25	25

[1] These ingredients were mixed in the above quantities and divided into two portions, each weighed out on 90 gm. of flour basis, before the fermentation process.
[2] The yeast was added to dough as a 25 ml. suspension in water.
[3] Hobart-Swanson mixer of 200 gms. flour capacity.
[4] Punching and molding were done according to the AACC standard hand method.

Results

The data for milk heated after reconstitution are given in Table II and Fig. 1. Six variables in duplicate were included in each day's baking. Two temperature variables were used each day, and were staggered so that each temperature variable was used with every other temperature variable an equal number of times. A total of 10 bakes (20 loaves) for each variable was made. The results were not corrected for inter-day variations. To each series of bakes there were added a no milk control and one in which the milk had received no heat treatment.

Two methods of grouping the data for statistical treatment were tried. In one method the amount of whey protein left undenatured by the heat treatment used was calculated as mg. of whey protein nitrogen per g. of milk solids, and grouped according to temperature-time variables used. In the other method this amount was subtracted from the value found for the original unheated milk, then converted to percentage of the total original whey protein, and grouped according to the percent denatured. Using the first method of grouping, the correlation coefficient between loaf volume and the amount of whey left undenatured by holding times of 5, 10, and 15 minutes at a given temperature was calculated. The temperature variables used were 70, 75, 77.5, 80, and 85°C. and the corresponding correlation coefficients were −0.055, −0.366, −0.764, −0.572 and −0.482, respec-

tively. There were 58 degrees of freedom for each coefficient. Consequently, for the 5% level of significance the coefficients needed to exceed the value of 0.26 while for the 1% level a value of 0.33 should be exceeded. All of the calculated coefficients exceeded the 1% level with the exception of the 70° temperature variable which was not significant at either level.

When all the data were grouped according to the percentage whey protein denatured, there was no significant linear correlation between

TABLE II

BAKING RESULTS FOR MILK HEATED AFTER RECONSTITUTION

Heat Treatment		Whey Protein Nitrogen		Loaf Volume[1]	
Temp.	Time	Undenatured	Denatured	Average	Standard[2] Error
(°C.)	*min.*	*mg./gm.*	%	*cc.*	*cc.*
85	5	1.77	79.6	839	7.0
	15	.87	90.0	880	6.4
	30	.64	92.6	880	8.0
80	5	4.14	52.1	810	9.8
	15	1.90	78.1	880	9.1
	30	1.06	87.8	882	12.0
77.5	5	5.84	32.7	772	7.2
	15	3.47	60.0	832	6.9
	30	1.99	77.0	859	7.5
75	5	6.81	21.4	784	8.1
	15	5.59	36.0	795	8.1
	30	3.99	54.0	821	11.6
70	5	7.78	10.2	788	8.2
	15	7.05	18.7	803	9.8
	30	6.22	28.2	801	7.7
No heat		8.67	0.0	841	5.9
No milk control				792	4.4

[1] Each average represents 10 series of bakes (20 loaves).
[2] Standard Error equals standard deviation of the mean.

this variable and loaf volume in the range below 50% denaturation. In the range where more than 50% of the whey protein had been denatured, the correlation was significant beyond the 1% level with a correlation coefficient of +0.581. Linear regression gave the following equation: Loaf volume = 1.72 (% whey protein denatured) + 724.

Group comparisons showed that the mean loaf volumes of bread made from milk without heat treatment were significantly greater than those made from milk heated at 70°C. for 5 minutes, 70° for 15

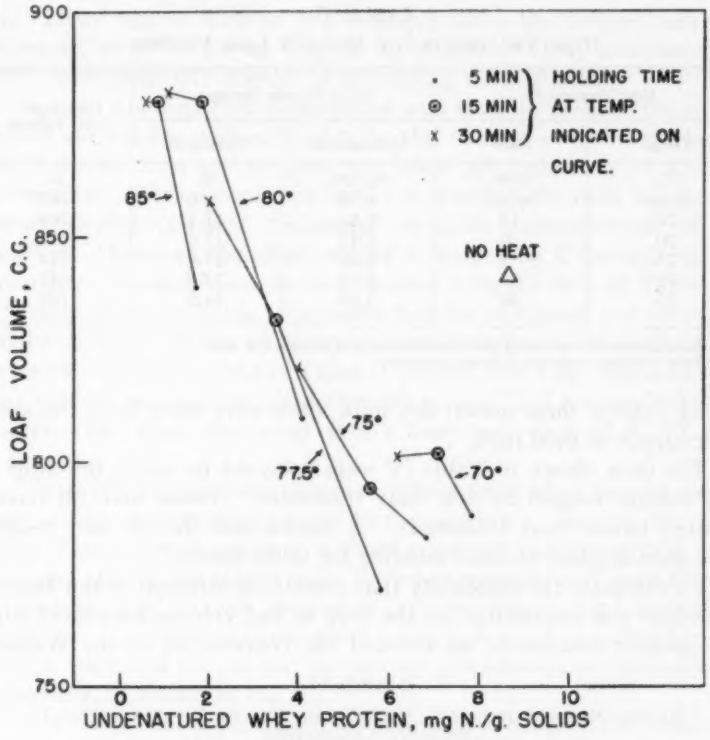

FIG. 1. Relation of undenatured whey protein to loaf volume.

minutes, 70° for 30 minutes, 75° for 5 minutes, 75° for 15 minutes, and 77.5° for 5 minutes.

To determine whether the same relationships existed when the milk was preheated before drying, the data shown in Table III were col-

TABLE III

COMPARISON OF HEAT TREATMENT BEFORE AND AFTER DRYING

Heat Treatment			Whey Protein Nitrogen		Average[1] Loaf Volume
Method	Temp.	Time	Undenatured	Denatured	
	°C.	min.	mg./gm.	%	cc.
None	—	—	6.60	0	824
Preheat	70	5	6.42	2.7	818
Preheat	80	30	1.77	73.1	896
Reconstituted	70	5	6.24	5.5	773
Reconstituted	80	30	1.05	84.1	917
No milk	—	—	—	—	794

[1] Each average represents 4 bakes (8 loaves).

TABLE IV

HEAT TREATMENTS FOR MINIMUM LOAF VOLUMES

Heat Treatment[1]		Whey Protein Nitrogen		Average[2] Loaf Volume
Temp.	Time	Undenatured	Denatured	
°C.	*min.*	*mg./gm.*	%	*cc.*
None	None	6.58	0	761
70	15	5.83	11.4	749
70	30	5.43	17.5	742
75	5	5.46	17.0	744
75	15	5.13	22.0	745
75	30	3.85	41.5	769

[1] Heat treatment conducted after reconstitution of sample No. 161.
[2] Each average represents 3 bakes (6 loaves).

lected. All of these nonfat dry milk solids were made from the same bulk supply of fluid milk.

The data shown in Table IV were collected to verify the drop in loaf volume caused by low heat treatment. These were all reconstituted before heat treatment. A mechanical sheeter and molder were used in place of hand molding for these results.

To eliminate the possibility that something inherent in our baking procedure was responsible for the drop in loaf volume associated with low preheat treatment, we enlisted the cooperation of the Western

TABLE V

BAKING RESULTS WITH 6% MILK SOLIDS BY THE WESTERN WHEAT QUALITY LABORATORY

Preheat Treatment		Whey Protein Nitrogen		Average Loaf Volume
Temp.	Time	Undenatured	Denatured	
°C.	*min.*	*mg./gm.*	%	*cc.*
No heat		6.80	0.0	855
77	0	5.73	15.7	800
77	5	4.78	29.7	812
77	30	3.15	53.7	848

Wheat Quality Laboratory, U.S.D.A., in making a few check bakes. (Table I, Laboratory B.) The milk was preheated before drying for the results shown in Table V.

Discussion of Results

In the range of heat treatment between 75°C. for 5 minutes and 80°C. for 30 minutes, there was a marked improvement in baking quality which was closely correlated with the denaturation of the whey protein. This is clearly shown in Fig. 1 plotted from the data of Table

II. There was no further improvement when the temperature was increased to 85°C. for as long as 30 minutes. Heat treatments at 70°C. were definitely inferior at all holding times studied.

A totally unexpected observation was that no preheat treatment before condensing and spray drying the milk gave definitely better loaf volumes than any temperature below approximately 77.5°C. for 15 minutes. This observation held true whether the milk was heated before drying (preheat treatment) or after reconstitution of the powder. However, the effect seemed to be greater if the heating was done after reconstitution, as may be seen from the data of Table III. The data shown in Table IV indicate that the minimum loaf volume is produced with milk heated enough to denature about 17% of the whey proteins. However, data of Table II showed that a minimum average loaf volume was secured when as much as 32.7% of the original whey proteins had been denatured with a heat treatment of 77.5°C. for 5 minutes.

These observations indicate that undenatured whey protein, *per se*, may not be the complete answer to the problem. There is the possibility that a small amount of heat treatment may activate some material closely associated with the whey protein, and that this activated material is then destroyed only by drastic heat treatment. However, after the minimum point is reached, there is a close correlation between loaf volume and the amount of undenatured whey protein remaining, as shown in Fig. 1.

Our results show that the minimum heat treatment for maximum loaf volume is 80°C. (175°F.) for 15 to 30 minutes. This heat treatment leaves less than 2 mg. of undenatured whey protein nitrogen per gram of nonfat dry milk solids and is in agreement with the results we secured on 35 samples of good and poor baking commercial samples (5). The above optimum heat treatment may be compared with several reports in the literature. Greenbank *et al.* (2) found that preheating to 95°C. for 30 minutes, or 85° for 2 hours, or 75° for 4 hours gave maximum loaf volumes. Grewe *et al.* (3) got a maximum loaf volume when the milk was preheated to 83°C. for 30 minutes. Skovholt and Bailey (9) found the baking quality to be about the same for preheating temperatures of 77°, 88°, or 96°C. with a time interval of 30 minutes; however, maximum absorption was secured only at the higher temperatures.

The recent work of Larsen, Jenness, and Geddes (7) indicated that maximum loaf volumes may be secured with heat treatment of 73°C. for 30 minutes, about 85°C. for 7 minutes, or 92°C. for 1 minute. These values are somewhat below what we consider optimum. One possible explanation is that since they did not include shortening in

their baking formula their loaf volumes are below ours for all heat treatments. They report considerably larger loaf volumes when no milk was included in the baking formula over those secured when 12% milk solids were added per 100 gms. of flour. We found, on the other hand, that the inclusion of 8% milk solids with no heat treatment raised the average loaf volume from 792 cc. to 841 cc., an increase of 6%, whereas if the milk had been properly heat treated the loaf volume averaged 880 cc., an increase of 11% over the no milk control. These values are shown in Table II and represent the average of 20 loaves each.

Acknowledgments

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OXIDATION OF AMYLOSE WITH HYPOCHLORITE AND HYPOCHLOROUS ACID[1,2]

C. H. HULLINGER AND ROY L. WHISTLER

ABSTRACT

Wheat and potato amylose in aqueous suspension on treatment with increasing concentrations of hypochlorite at pH 7 show a decrease in viscosity and in the rate of retrogradation and an increase in the quantity of aldehyde and carboxyl groups. About one-half of the introduced carboxyl groups are of the uronic acid type.

Products which result when potato amylose samples are treated with equal amounts of oxidant in the form of hypochlorite and hypochlorous acid at pH values from 2 to 12 have similar viscosities and uronic acid content. As the pH of the oxidation is increased the comparative aldehyde content of the products decreases, while the carboxyl content increases.

The modification of starch by the action of hypochlorites has been known and applied in industry for some time, but the nature of the reactions involved is still obscure.

Jambuserwala and Kanitkar (6) treated whole starch with neutral hypochlorite solutions of different concentrations and observed in each case an increase in reducing value and acidity along with a decrease in viscosity. Similar results are reported for alkaline hypochlorite oxidation (7, 13). Fletcher and Taylor (5), however, observed that alkaline oxidation of β-amylose (crude amylopectin) decreased the reducing value and had little effect on the viscosity.

Staudinger and Jurisch (17) stated that the most important action of hypochlorite on cellulose is a splitting of the molecule near the center of the chain. Brissaud (2) reported the formation of small amounts of gluconic, glucuronic, and saccharic acids when cellulose is treated with hypochlorite. Under neutral conditions an increase in the extent of hypochlorite oxidation is accompanied by an increase in reducing power and acidity. Davidson and Nevell (4) observed the presence of carbonyl and carboxyl groups in cellulose treated with hypochlorite, as did Meesook and Purves (12).

The work reported herein was carried out to ascertain the effect of oxidant concentration and pH upon the hypochlorite reaction.

Marsh (8) reported that when cellulose is treated with chlorine solution the highest carboxyl content is obtained when the oxidation

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[2] Department of Agricultural Chemistry, Purdue University, Lafayette, Indiana.

takes place at pH 10–11 while at pH 4, the highest reducing values are found. At pH 10 the chlorine is present largely as hypochlorite ions, while hypochlorous acid is the major component at pH 4.

Materials and Methods

Amyloses were prepared by butanol fractionation (15, 16) as previously described (19).

Wheat amylose in a 2% suspension and potato amylose in a 1% suspension in water were oxidized by sodium hypochlorite (1) at the levels shown in Tables I and II. Potato amylose in a 1% suspension

TABLE I

ANALYSIS OF WHEAT AMYLOSE OXIDIZED WITH HYPOCHLORITE AT PH 7

Equivalent of Oxidant Consumed per Mole of Anhydroglucose Units	0.5	0.2	0.1	0.04	0.02	0.01
Total Carboxyl[1]	0.065	0.036	0.025	0.014	0.008	0.012
Uronic Acid Carboxyl[1]	0.036	0.013	0.004	0.002	0.001	0.001
Aldehyde[1]	0.042	0.022	0.005	0.003	0.003	0.002
% Retrogradation	28	65	92	97	98	98
Specific Viscosity (1.0% solution)	0.087	0.127	0.223	0.439	0.606	0.626

[1] Moles of functional groups per mole of anhydroglucose.

TABLE II

ANALYSIS OF POTATO AMYLOSE OXIDIZED WITH HYPOCHLORITE AT PH 7

Equivalent of Oxidant Consumed per Mole of Anhydroglucose Units	1.0	0.5	0.18	0.1	0.04	0.02	0.01
Total Carboxyl[1]	0.084	0.053	0.028	0.017	0.008	0.008	0.007
Uronic Acid Carboxyl[1]	0.028	0.016	0.015	0.011	0.001	—	0.003
Aldehyde[1]	0.042	0.049	0.016	0.015	0.005	0.006	0.004
% Retrogradation	18	35	90	95	97	99	96
Specific Viscosity[2] (1.0% solution)	0.076	0.084	0.228	0.239	0.683	1.001	1.830

[1] Moles of functional groups per mole of anhydroglucose.
[2] Viscosity of original amylose was 4.08.

in water was oxidized by hypochlorite at the concentration and pH levels shown in Table III. Amylose concentration in the paste was determined by suspending a known amount of paste in water and drying aliquots of the suspension mixed with filter cel *in vacuo* at 100°C. over night. All oxidations were carried out at room temperature.

For oxidations at pH 7, a known amount of amylose-butanol paste was dispersed in a boiling mixture of 1,500 ml. of water and 500 ml. of Clark and Lubs buffer solution. (Chapin (3) reports that phosphate, at concentrations below 0.25 *M*, has little effect on the action of hypo-

chlorite.) The suspension was heated in a boiling water bath until the odor of butanol had disappeared. It was then cooled and the volume adjusted to 2 l. The calculated amount of hypochlorite was added and the pH brought immediately to 7 with dilute hypochloric acid. The reaction mixture was placed in a stoppered bottle and agitated continuously. The pH was adjusted at frequent intervals to prevent deviations of more than one-half pH unit. Oxidations at pH 7 were usually complete in four days while seven to ten days were required at the other hydrogen ion concentrations.

When all the hypochlorite had disappeared, the mixture was dialyzed in cellophane bags for 48 hours against 40 l. of distilled water. After dialysis, one-third of the reaction mixture was removed for retrogradation and viscosity studies (19). The remainder was electrodialyzed between parchment membranes until its conductivity ap-

TABLE III

ANALYSIS OF POTATO AMYLOSE OXIDIZED BY 0.1 EQUIVALENT OF HYPOCHLORITE PER MOLE OF GLUCOSE ANHYDRIDE

pH of Reaction	2	3	5	7	9	10.5	12
Total Carboxyl[1]	0.007	0.014	0.013	0.017	0.014	0.017	0.026
Uronic Acid Carboxyl[1]	0.005	0.011	0.009	0.011	0.005	0.010	0.015
Aldehyde[1]	0.012	0.023	0.012	0.015	0.012	0.006	0.006
Specific Viscosity[2] (1.0% solution)	0.293	0.278	0.323	0.239	0.286	0.378	0.347

[1] Moles of functional groups per mole of anhydroglucose.
[2] Viscosity of original amylose was 4.08.

proached that of distilled water. The amylose in this portion was analyzed for uronic carboxyl (20), total carboxyl (10, 11, 18) and aldehyde groups (9). Since the method used in the determination of aldehyde groups is probably not quantitative for modified polysaccharides, the results obtained are of comparative value only.

For the oxidation at various hydrogen ion concentrations, the same amount of dibasic phosphate was used but the pH was adjusted with hydrochloric acid or sodium hydroxide to the desired value. Except that no retrogradation measurements were made, these samples were analyzed in the same way as the samples oxidized at pH 7.

Results and Discussion

Wheat and potato amylose samples treated with hypochlorite in neutral solution decrease in viscosity as the degree of oxidation increases (see Tables I and II). Also the rate of retrogradation of potato amylose first increases slightly and then decreases with increased amounts of oxidant in a manner similar to that for amylose depolymerized by acid hydrolysis (19). This seems to indicate that depoly-

merization results from neutral hypochlorite oxidation of amylose. In addition, the wheat and potato amylose samples treated with hypochlorite in neutral solution show an increase in aldehyde and carboxyl groups per anhydroglucose unit. The uronic acid group accounts for about one-half to one-third of the total carboxyl content.

The ratio of end groups (aldehyde plus carboxyl other than uronic) to anhydroglucose units approaches a limit of about 1 to 12 with increasing amounts of hypochlorite. This limit may be due to the method used in preparing the samples for analysis. Dialysis in cellophane and electrodialysis in parchment (designed to remove inorganic ions) also removes carbohydrate molecules of less than six to eight anhydroglucose units in length, since these molecules also can pass through the membrane.

The effect of pH levels between two and twelve on the hypochlorite oxidation of potato amylose is shown by Table III. The carboxyl content increases and the aldehyde content decreases with ascending pH. No significant change is observed in the uronic acid content, the viscosity, or in the total number of endgroups per unit weight of potato amylose.

While the yields of both aldehyde and carboxyl groups are affected by the hydrogen ion concentration, the degree of depolymerization appears to be the same at all the pH levels investigated.

It should be noted that at pH 2, the concentration of free chlorine is 31% of the total available chlorine (14), so the effects reported at that hydrogen ion concentration cannot be attributed entirely to hypochlorite and hypochlorous acid.

Acknowledgment

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A NOTE ON THE APPARENT REVERSAL OF THE OXIDATION OF CAROTENOID PIGMENTS DURING MIXING OF MACARONI DOUGHS IN 40% ALCOHOL[1]

G. N. IRVINE[2] and C. A. WINKLER[3]

ABSTRACT

The reversal of the pigment oxidation during mixing of macaroni doughs in 40% alcohol previously reported has been confirmed. A possible mechanism is outlined which accounts for this phenomenon.

In a previous paper (1) evidence was presented which indicated that during mixing of macaroni doughs in 40% alcohol, the reaction responsible for the oxidation of the carotenoid pigments in the dough reversed after about 1½ minutes of mixing, and more prolonged mixing restored pigment to the system. Not only have several subsequent experiments confirmed this behavior, but evidence has now been obtained to indicate that the results are not due merely to an increase in extractability of the pigment. The present note about this behavior, suggesting a possible mechanism which could account for the observed results, might be of some interest, particularly in view of the frequently postulated role of the carotenoid pigments as oxygen carriers in photosynthesis.

The methods used in this work have been outlined in a previous paper (1). A typical reaction curve showing the variation of residual pigment content in the dough with mixing time is shown in Fig. 1. To ensure that no interfering substances were causing changes in the absorption spectra of the residual pigment extracts, complete spectra of all the extracts were obtained using a Beckman Spectrophotometer. In all cases the spectra were normal and the increasing pigment content with increasing mixing time was readily apparent from the absorption curves.

Reversal of the type shown in Fig. 1 might be due to the fact that prolonged mixing in 40% alcohol renders the pigment more extractable. To check this possibility, a dough was mixed in a 0.002 *M* solution of alpha naphthol in 40% alcohol. Alpha naphthol in this concentration prevents oxidation of the pigment over the initial portion of

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[2] Chemist, Grain Research Laboratory, Winnipeg, Manitoba.
[3] Professor of Chemistry, McGill University, Montreal, P. Q.

the reaction curve, while beyond this point the 40% alcohol completely inactivates the enzyme and prevents oxidation. Thus, mixing in this medium should prevent any oxidation of pigment. It follows that if the pigment is rendered more extractable on mixing in 40% alcohol,

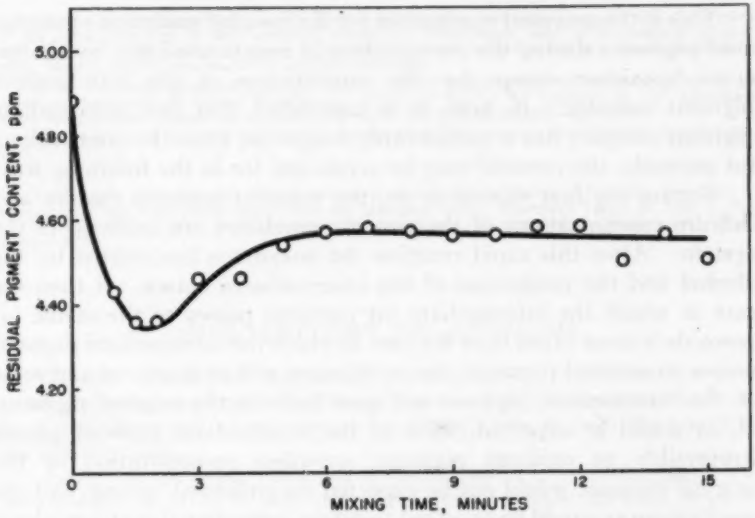

FIG. 1. Effect of mixing time on pigment content of macaroni doughs mixed in 40% alcohol.

prolonged mixing in the alpha naphthol—40% alcohol mixture should result in more pigment being extractable after longer mixing periods than was originally extractable from the semolina (t = 0 min.). It is apparent from the results in the following table that there is no increase in extractable pigment with mixing time. Accordingly, it is suggested that a genuine reversal of the reaction occurs on prolonged mixing in 40% alcohol in the absence of alpha naphthol.

Mixing time, minutes	0	1	3	5	7	9
Residual pigment, p.p.m.	4.01	3.92	3.95	3.97	3.91	3.92

The following mechanism will account for the observed reversal of the reaction:

$$\mathrm{L} + \mathrm{O_2} + \mathrm{U.F.} \rightleftharpoons \mathrm{L} - \mathrm{O_2} - \mathrm{U.F.} \rightleftharpoons \mathrm{L} + \mathrm{I.U.F.} \rightarrow \mathrm{U.F.P.}$$

$$+$$

$$\text{pigment}$$

$$\Uparrow\Downarrow$$

$$\text{intermediate pigment complex}$$

$$\downarrow$$

$$\text{oxidized pigment}$$

Where L is Lipoxidase,

U.F. is unsaturated fat,
I.U.F. is intermediate fat peroxide, and
U.F.P. is unsaturated fat peroxide.

This is the accepted mechanism for the coupled oxidation of carotenoid pigments during the peroxidation of unsaturated fats by the enzyme lipoxidase except for the introduction of the intermediate pigment complex. If, now, it is postulated that this intermediate pigment complex has a considerably longer life than the intermediate fat peroxide, the reversal may be accounted for in the following way.

During the first minute or so, the reaction proceeds rapidly and definite concentrations of the two intermediates are built up in the system. After this rapid reaction the enzyme is inactivated by the alcohol and the production of the intermediates ceases. If then the rate at which the intermediate fat peroxide passes to the stable fat peroxide is more rapid than the rate at which the intermediate pigment passes to oxidized pigment, the equilibrium will be displaced and some of the intermediate pigment will pass back to the original pigment. If, as would be expected, some of the intermediate pigment passed irreversibly to oxidized pigment, complete reconstitution of the original pigment would not be expected on prolonged mixing, and the reaction curve would be expected to attain a steady value at some level below the original concentration. This is, in fact, the experimental behavior (Fig. 1).

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Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B+C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see
Cereal Chem. **27**: 519–520 (1950).

Sterling-Winthrop Research Institute serving Sterwin Chemicals Inc.

Sterwin

HELPS YOU PRODUCE BETTER FOOD PRODUCTS

Easily . . . Accurately . . . Economically

STERWIN CHEMICALS, backed by the extensive manufacturing and research facilities of the entire Sterling Drug organization, is continually striving to supply food processors with modern products and methods that will aid in the production of *better* food products *easily*, *accurately* and *economically*.

Contributions to the Food Industry by Sterwin products have been many. Outstanding among them are the following famous products . . . each a *first* in its field:

FIRST — BREAD-ENRICHMENT TABLETS

B-E-T-S® Enabled bakers to comply promptly with War Food Order No. 1. This tablet method has made enrichment in the bakery both practical and economical . . . saving bakers many millions of dollars.

FIRST — LIGNIN VANILLIN MADE IN U. S. AMERICA

ZIMCO® Pure Lignin Vanillin U.S.P. Made by world's largest Vanillin manufacturer. Preferred by leading flavor suppliers and manufacturers of confections, ice cream, baked goods, soft drinks and other foods.

FIRST — LOW-ASH FOOD ENRICHMENT MIXTURE

VEXTRAM® The original starch base enrichment mixture. Keeps market value of enriched flour up by keeping the ash content low. Free-flowing . . . uniform . . . stable.

FIRST — SYNTHETIC VITAMIN D_3

TRIDEE® The form of Vitamin D which has the greatest antirachitic value in poultry. D_3 is also used for fortification of evaporated milk and other food products.

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ROCCAL® A powerful germicide that may be used for every equipment sanitizing job throughout the Food Industry.

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Distributor of the products formerly sold by Special-Markets-Industrial Division of Winthrop-Stearns Inc., and Vanillin Division of General Drug Company.

CONGRATULATIONS, BAKERS_

15 MILLION FAMILIES will be reached by this double-page spread heralding the 10th Anniversary of the Bread Enrichment Program.

Featuring congratulatory messages from 10 outstanding nutrition leaders, the advertisement will appear in *Saturday Evening Post,* March 31; in the April *Ladies' Home Journal;* in *Look,* April 10; and in the May *Woman's Home Companion.*

Valuable tie-in material is available to bakers from the Bakers of America—who are sponsoring the advertisement.

ABC TO BROADCAST ENRICHMENT FILM ON NATION-WIDE TELEVISION NETWORK

● In March—in cooperation with The Fleischmann Division of Standard Brands—the American Broadcasting Company will present "The Modest Miracle" over its coast-to-coast television network, reaching an audience of millions of bakery customers.

This famous film—produced by the makers of Fleischmann's Yeast in behalf of the baking industry—has been shown in more than 3,000 theaters and thousands of schools, bringing the dramatic story of Bread Enrichment to millions of American people.

See "The Modest Miracle" on ABC-Television in March—and use it yourself to tie in with Enrichment's 10th Anniversary. Your Fleischmann man will be glad to arrange a showing.

Consult your local newspaper for time and date. Ask your Fleischmann man too for other Enriched Bread merchandising tie-up suggestions.

SCENES FROM

Dr. Christian Eijkman, sent by the Dutch to Java in the 1890's, attempts to stop the epidemic of beri-beri that is ravaging the island's native population.

on the 10th Anniversary of BREAD ENRICHMENT!

The Makers of Fleischmann's Yeast Join the Nation's Nutrition Leaders in Saluting the Achievements of this Program in Building a Healthier, Stronger America.

• During March America celebrates the 10th Anniversary of one of the most dramatic projects ever undertaken in the field of human nutrition... the Bakers of America's voluntary Bread Enrichment Program.

The impressive accomplishments of this cooperative venture in bringing better health—and happier lives—to millions of American people will be the subject of intensive promotion throughout the baking industry.

Twin keynotes of this important observance will be:

1. **The Bakers of America** advertisement described in detail on the opposite page, and

2. **The Nation-Wide Broadcast**—over ABC-Television—of "The Modest Miracle," Fleischmann's widely-heralded documentary film featuring the dramatic discovery of Vitamin B_1.

AVAILABLE FOR FREE SHOWINGS

This timely, informative film is available to Bakery Associations and individual bakers for use at conventions, association meetings, sales and production meetings and with schools, colleges, women's clubs, etc. To arrange a showing—just ask your Fleischmann man. *Use "The Modest Miracle"—to tie in with the big celebration of the 10th Anniversary of the Bread Enrichment Program.*

THE MAKERS OF FLEISCHMANN'S YEAST

"THE MODEST MIRACLE"... on ABC-TV IN MARCH

Dr. Casimir Funk discovers that yeast contains the same health-giving substance earlier found in rice bran. He gives this substance a name—"vitamine."

A native Filipino woman's plea for help for her dying baby moves Dr. R. R. Williams, an American scientist, to try an extract made from the brown coating of rice.

Announcement is made in 1941 that America's bakers and millers will co-operate with the National Nutrition Program by producing "enriched" bread and flour.

NOW...GREATER STABILITY OF THIAMINE IN FLOUR WITH

Thiamine Mononitrate Merck

CEREAL chemists have welcomed with enthusiasm the introduction of Thiamine Mononitrate, now available in the full line of Merck Vitamin Mixtures for Flour Enrichment.

Thiamine Mononitrate is a more stable form of thiamine, assures a more complete retention of vitamin B_1 content in enriched flour—even under adverse conditions of temperature and humidity during shipping and storage.

Leading cereal chemistry laboratories participated in the thorough investigation of Thiamine Mononitrate in enriched flour. Since then, extensive commercial experience and rigorous field tests have confirmed that Thiamine Mononitrate represents a signal improvement in flour enrichment; that it offers a degree of protection hitherto not available.

Each ounce contains 369 milligrams Thiamine Mononitrate

NO CHANGE IN LABELING REQUIRED

Thiamine Mononitrate meets every requirement for thiamine under the Federal Standards of Identity for enriched flour. No need for change in the labeling on bags or packages to comply with Federal requirements when you use these improved Merck Vitamin Mixtures.

Each ounce contains 738 milligrams Thiamine Mononitrate

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MERCK ENRICHMENT MIXTURES

For Your Vitamin Analyses

THE COMPACT COLEMAN PHOTOFLUOROMETER

NOW USED BY HUNDREDS OF LABORATORIES

*** Representative Photofluorometer Users:**

Abbott Laboratories(4)	Univ. of Arkansas	Inst. of Paper Chemistry
American Can Company	Armour & Company(8)	Swift & Company(5)
American Inst. of Baking	Quaker Oats Company(3)	Western Condensing(17)
American Medical Assoc.	Univ. of Illinois(6)	Univ. of Wisconsin(4)

For ALL Fluorescence Tests

The compact Coleman Photofluorometer is in use in hundreds of laboratories and should be specified where many tests are made daily and also where low concentrations of vitamins B1 and Ba, Atabrine, etc., are encountered.

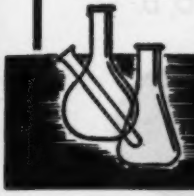
Removable filters are used for selection of proper mercury line and screening of photocell. Light source is AH-3 mercury lamp and photocell is used in conjunction with a high gain electronic amplifier.

No. CC-7196. Coleman Model 12B Electronic Photofluorometer, Complete with batteries, AH-3 lamp, amplifier tube, auto-transformer for operating at 110 Volts 60 cycle, one dozen, marked cuvettes. Without filters. Each $320.00

No. CC-B1. For 365 mu. Hg. line. Corning No. 5874. Each 11.00
No. CC-B2. For 436 mu. Hg. line. Corning No. 3389 1/2 5113. Each 12.00
No. CC-PC1. Absorbs below 425 mu. No. 3398 and No. 4308. Each 12.00
No. CC-PC2. Absorbs below 525 mu. No. 3486. Each 8.00
No. CC-7195-WS Sta-Bal Voltage Regulator. Attaches to 110 volt, A.C. line and provides steady voltage required by the mercury lamp. Each 85.00

Filters are for selecting the proper mercury line and also for shielding the photocell so that stray light is not reflected onto the cell. For Thiamine, the No. CC-B1 or CC-B1S filters are used to select the mercury line and the No. CC-PC1 filter protects the photocell. Similarly the No. CC-B2 and CC-PC2 filters are used for Riboflavin.

An Authorized Coleman Dealer



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WILKENS-ANDERSON CO.

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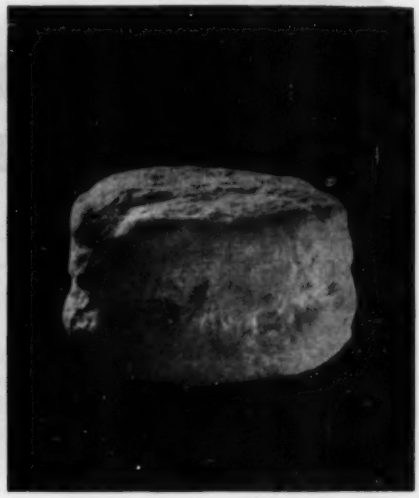

V-90* Makes the Difference . . . this difference makes SALES for you!

That really good-looking, good-eating *biscuit* on the left is the kind your customers have come to expect of self-rising flour everytime . . . the kind that *only* self-rising flour made with S-L-O-W acting V-90 phosphate can assure! This striking difference in baking performance helps you increase and *hold* profitable self-rising flour business!

V-90®

THE ONLY PHOSPHATE CRYSTAL WITH A "RAIN COAT"*

*Coated Anhydrous Monocalcium Phosphate

REMEMBER . . .

Long, safe shelf life for your self-rising flour is assured when it is made with slow-acting V-90*. This special protection is a plus feature which you gain because of the unique coating of each V-90 crystal. Your brand enables the housewife ready to deliver that superior kind of baking performance she's learned to expect!

VICTOR CHEMICAL WORKS

141 West Jackson Blvd. • Chicago 4, Illinois

STIMULATES SELF-RISING SALES . . . STIMULATES SELF-RISING SALES . . .

Service When It Counts

Every so often, we all need assistance and need it badly, to furnish the answers to many of our problems. In the Flour and Cereal Industry, N-A's Flour Service Division has been supplying practical, down-to-earth solutions to many of the industry's problems for over 25 years. Using its broad background of experience in the manufacture of time-tested products for maturing, bleaching, and enriching, the N-A Flour Service Division is ready to put its nation-wide service organization and extensive laboratory and research facilities at your service. They will work closely with your own staff and consultants to produce a concrete, individual solution to fit your particular needs.

For more details, call your nearest N-A Representative today and find out how this service can work profitably for you.

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